Addendum to the PAL User Manual PAL DLW Option Installation and Operation

DLW: Dynamic Load & Wash

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Original Instructions

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A. Safety Information

Declaration of Conformity

Please refer to the Declaration of Conformity sheet enclosed with the instrument.

General Considerations

Changes or modifications to this unit not expressly approved by the party responsible for compliance could nullify the user's authority to operate the equipment. The user should be aware that using the equipment in a manner not specified by the manufacturer may compromise its safety features .

Electrical Hazards

Every analytical instrument has specific hazards, so please read and comply with the precautions as described in the corresponding PAL User Manual.
Only use fuses of the type and current rating specified.
Do not use repaired fuses and do not short-circuit the fuse holder.

Other Hazards

To avoid injury while operating the PAL System, keep your hands away from the syringe.

For detailed Safety Information, see the additional warnings in the corresponding PAL User Manual.

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Commonly Used Symbols

	Caution, or refer to User Manual
	Caution, Risk of Needle-Stick Puncture
	Caution, Hot Surface or High Temperature
	Direct Current
	Alternating Current
	Protective Conductor Terminal, Ground
	Fuse
	Electrical Power ON Used with Main PAL Power Supply
	Electrical Power OFF Used with Main PAL Power Supply
	Electrical Power ON for only part of the System Used with Optional Device(s)
	Electrical Power OFF for only part of the System Used with Optional Device(s)
	Caution, Risk of Electrical shock (high voltage)
	Disposal, Do not dispose in municipal waste. Follow local waste regulations to reduce electrical and electronic waste (WEEE).

Table 1. Commonly Used Safety Symbols.

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D. How to Use this Manual

note *The PAL System must be installed and properly set up before the DLW Option instructions can be implemented.*

This manual is divided into the following sections:

- Installing the DLW Option
- Operating the DLW Option
- Troubleshooting
- Replacing Parts
- Maintenance
- Limitations
- Appendices

The "DLW Installation Instructions" are intended for frequent PAL users or new users who are already experienced at using automated systems to run existing analytical procedures.

The Appendices provide information on PAL DLW macros for multiple injection valve combinations, and on spare parts.

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E. PAL DLW Option Installation

1. General System Overview

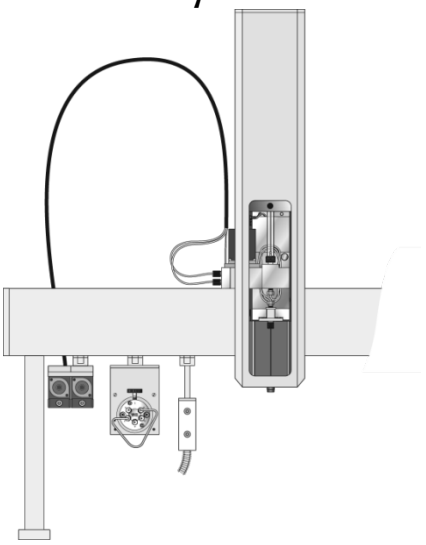

Figure 1. PAL DLW Option General System Overview

DLW: Dynamic Load & Wash

The DLW Option represents a new concept in wash stations, combining an injection cycle with wash steps. The combination of both injection and wash steps – usually separate procedures – minimizes cycle time and carry-over.
The two characteristic features of the DLW Option are:

- The sample solution never comes in contact with the syringe itself; it is held "sandwiched" in a so-called Holding Loop.
- Wash solvents are pumped from the rear to the front of the DLW system, to intensely purge all critical surfaces which have come in contact with the sample.

The DLW option consists of two self-priming micro pumps (mounted on a dedicated bracket), the wetted parts are Ryton PPS and Kalrez (FFPM). The "IN" ports are connected to the wash solvent bottles and the "OUT" ports are connected to the DLW manifold, which is part of the dedicated DLW Syringe Holder assembly. A "Holding Loop" separates the syringe and the DLW actuator to avoid the sample coming in contact with these parts.

The syringe and Holding Loop are preloaded with Wash Solvent #1 at the start. The sample is picked up and remains separated from Wash Solvent #1 by an air gap. After loading the loop and injection, Wash Solvent #1 is pushed into the system, followed directly by Wash Solvent #2 to flush the critical valve paths.

The DLW Syringe Assembly is then moved to the wash station for further cleaning steps and to prepare the Syringe and Holding Loop for the next cycle.
Further details are given in section G "Operation", point 5 "Cycle Step-by-Step".

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1.1. Specifications

Part Number:	PAL DLW Option
• DLW Pumps:	Two pcs. solenoid diaphragm pump, self-priming
- Flow rate range:	100 mL/min at atmospheric pressure (Pump itself, no connections to HPLC system)
- Wetted parts	Pumps: Ryton PPS and Kalrez (FFPM)
• Transfer Tubing Kit:	Two pcs. 1/8" PFA tubes including connection fittings
• Solvent Reservoir:	1000 mL borosilicate glass including Glass Filter,40 µm pore size
• DLW Wash Station:	Wash Station with two solvent ports and one Waste port. Two additional horizontal Waste inlet ports in front to be combined with Valve waste port.
- Wetted parts:	- Wash Station block: PVDF - Inserts: sst 1.4404 - Connections at bottom of inserts: PEEK - Waste Tube: Polyethylene (PE)
• DLW Syringe Assembly:	
o Syringe:	Gastight Syringe 100 µL with Valflon tip
- Wetted parts:	Glass, PTFE and Valflon
o Syringe Needle:	Gauge 22 (OD 0.72 mm/ID 0.41 mm) Length: 51 mm, PTFE Seal
o DLW Actuator/ Solenoid:	2/2-way flipper solenoid valve
- Wetted parts:	Body: PEEK; Seal material: FFKM (Simriz)
o DLW Manifold adapter assembly	Solvent selector module and Syringe
- Wetted parts:	Block: sst 1.4435, Perfluor (O-ring)
o Holding Loop:	FEP Tubing OD 1/16", ID 0.50 mm", length 55 cm Loop Volume: 108 µL
o DLW Flow Diverter:	Tube dimensions: - Gauge 22 (OD 0.72 mm/ID 0.41 mm) - Length: 20.5 mm
- Wetted part:	stainless chromium steel
o DLW Needle Holder Assembly:	
- Wetted part:	sst 1.4435

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1.2. Hardware Requirements

The PAL DLW option can be used with HTS-*xt* or HTC-*xt* PAL instruments in combination with one or several injection valves of the following types: VICI DCxxWK (conical rotor), VICI Cheminert, or Rheodyne ultra high pressure valves.

The PAL Injection Unit needs to be modified in order to install the DLW Option. Details are described in Section E, point 2.2 “Preparation of Injection Unit for DLW Option”.

1.3. Software Requirements

The PAL DLW Option can only be operated with PAL-*xt* Firmware version 4.1.X. or higher.
It can be controlled by the Cycle Composer or any data system that controls the PAL using PAL ICC (e.g. Analyst, ChemStation, Empower, EZChrom, MassLynx, Xcalibur).

For details see Section F, point 1, “PAL Firmware and Firmware Object Installation for PAL DLW Option” and Section K, “Limitations”.

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2. Installation of the DLW Option

2.1. Unpacking the Components

The PAL DLW option is shipped in one box. Check for the following items:

1. DLW Pump Module
2. DLW Syringe Needle
3. DLW Syringe
4. DLW Plunger Holder
5. DLW Flow Diverter (installed in the DLW Syringe Assembly)
6. DLW Syringe Holder Assembly
7. CD-ROM
8. Wash Station cable to PAL System
9. DLW Tubing Kit
10. DLW Holding Loop (installed in the DLW Syringe Assembly)
11. Needle Guide Length Tool
12. DLW Wash Station incl. Waste Tube
13. 2 pcs. Solvent Bottle Transfer Line including Glass Filter, Solvent Inlet, 40 µm pore size
14. 2 pcs 1 liter Solvent reservoir bottles

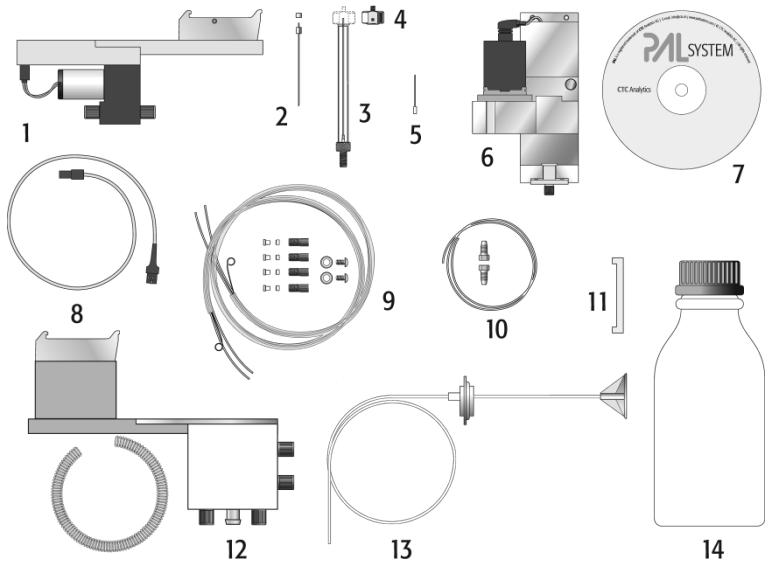

Figure 2. PAL Active Wash Station Components.

Figure 1 illustrates how the various DLW components as shown in Figure 2 are mounted on the PAL System.

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2.2. Preparation of the Injection Unit for the DLW Option

note

*New PAL **-xt** Systems equipped with a PAL DLW Option are already configured and ready for use. All the points described below are not applicable for new systems. For all field upgrades, make sure the instructions given below are followed.*

For field upgrades only:
The injection unit – the Z-axis – must be prepared in order to install the DLW option. The upper needle guide must be removed and the lower needle guide must be lowered to adapt to a needle length of 73 mm. The standard setting for the needle guide is 50.5 +0.5 mm.

Detailed descriptions for these maneuvers are given in the PAL Service Manual. A brief outline is provided below.

1. Remove the Injection Unit from the Y-axis.

2. Open the top cover to disconnect the long tension cord.

3. Pull out the slider to access the "Anti-Twist Device" of the upper needle guide. Open the Torx screw of the device.

4. Pull out the slider to access the "Mechanical stop for Needle Guide". Carefully open the Torx screw of the stop.

5. Pull out the lower needle guide assembly, until the Upper Needle Guide can be removed.

The new Upper Needle Guide, designed for use in combination with the PAL DLW Option, has a longer rod. This rod length is critical if the new version of the Upper Needle Guide is used, and in cases where the PAL System is retrofitted to standard liquid injection using a Wash Station other than the PAL DLW Option.
The longer rod would touch an electronic component from the Z-axis PCB. Therefore, the system must be adjusted to avoid this problem with the longer needle guide length. See Figure 3.

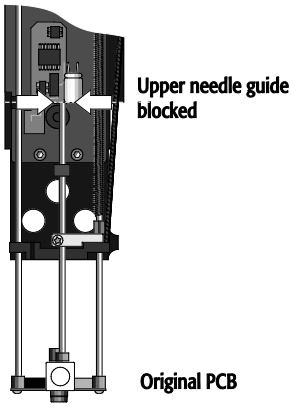

Figure 3. New Upper Needle Guide touching electronic Components.

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Starting with shipments March 2010 onwards for the PAL Z-axis with PNo. MZ 01-00F, a modified PCB was installed (PNo.: APR Head, Revision 'KM'). If this board is installed, the new type of Upper Needle Guide with the longer rod can be installed without any restrictions. See Figure 4.

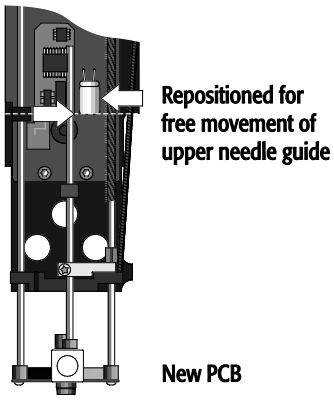

Figure 4. New Upper Needle Guide installed in Models with the modified PCB.

If the older version of the Z-axis PCB is installed, Revision 'KL'. We advise you to store the original Upper Needle Guide assembly for future use.

Replace the current Upper Needle Guide with the new version containing the longer rod.

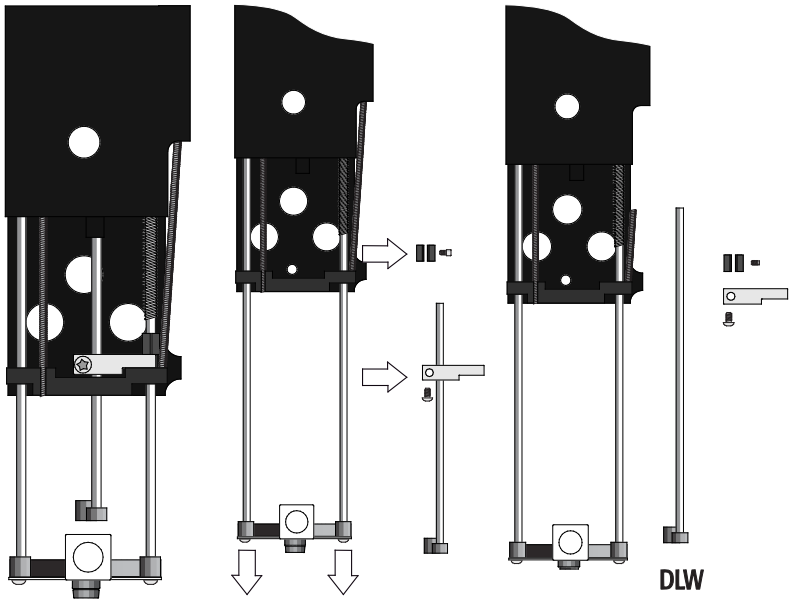

Figure 5. Preparing the Injection Unit for the DLW Option.

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6. The needle length has to be re-adjusted to 73.0 mm. A tool is provided with the DLW option, the “DLW Needle Length Guide Tool”. Insert the tool in one of the guide bars as shown in Figure 4 below.

7. Tighten the mechanical stop for the needle guide securely.

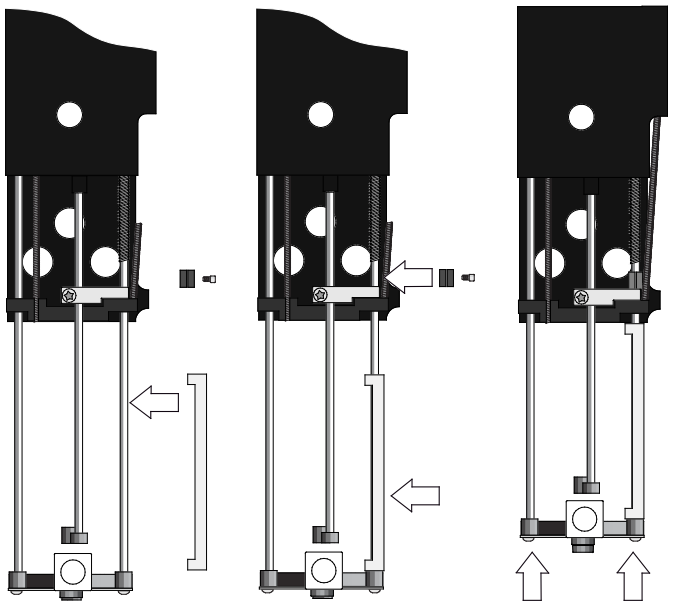

Figure 6. Adjusting the Needle Guide to Needle Length for the DLW Option.

8. Insert the syringe slider into the Z-Tube and attach the long tension cord to the top cover.

9. Reinstall the Injection Unit on the Y-Axis.

note

Figure 6 illustrates the position of the mechanical stop for the needle guide, shown outside the unit for illustrative purposes. When assembled, this part is mounted to the rod. This illustration emphasizes the fact that the stop must first be opened and then fixed again.

10. Adjusting the Upper Needle Guide is illustrated in Figure 7. This position was found to be ideal for changing the syringe needle without bending it at the Upper Needle Guide.

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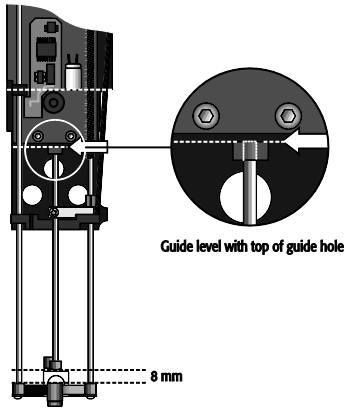

Figure 7. Adjusting the Upper Needle Guide.

2.3. Mounting the Pump Holder

The pump holder should always be mounted on the far left of the X-axis. The solvent lines are connected to the holder. Moving across the pump holder with the Z-axis could kink the stabilizing wire. To install the holder, we recommend dismounting the drive from the PAL X-axis.

1. Locate the pump holder and the two supplied Torx screws.

2. Attach the holder to the X-axis as shown below, in Figure 8.

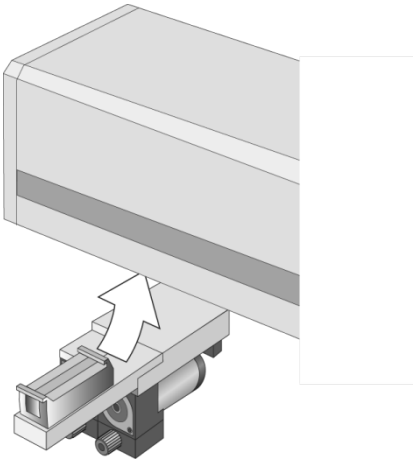

Figure 8. Attaching the Pump Holder Bracket to the X-Axis.

3. The Solvent line assembly is not preinstalled at the factory. The correct mounting is shown in Figure 9 below.

   Select the end of the of the guide wire with the longer tubing ends and connect the guide wire with the screw M4 x 8 and the corresponding serrated lock washer to the back side of the module. Note the guide groove, which orients the wire.

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note

Always install the DLW Tubing (from the kit) to the pump module first. The other end, connected to the DLW Syringe Assembly, should be connected while the assembly is not yet installed onto the injection unit.

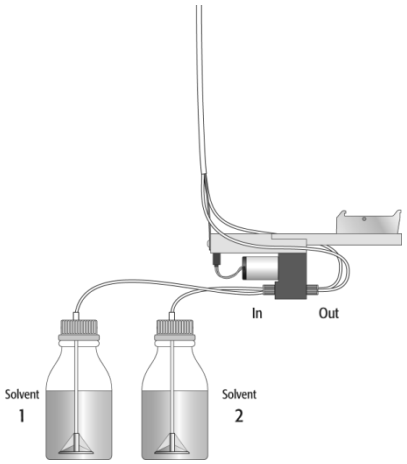

Figure 9. Installing the Solvent Tube Assembly to the Pump Holder.

4. Connect the solvent tubing of the DLW Tubing kit to the pump module, using the pre-mounted nut. Maintain this order: Solvent 1 connected to the left, and Solvent 2 to the right pump module, when viewed from the front of the PAL system. This avoids any confusion when refilling or changing solvents.

5. Connect the solvent lines from the solvent reservoir bottle to the "Inlet" connectors of the pump module.

6. The electrical connections from the pumps to the PCB sockets, mounted on the bracket, are done at the factory.

7. Insert the cable provided into the middle socket, between the other two sockets on the PCB. For details see Figure 10.

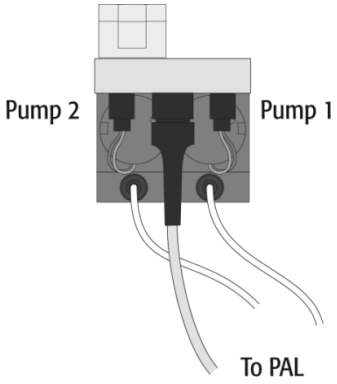

Figure 10. Electrical Connections at the Pump Holder Bracket.

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8. The other end of the cable is connected to the "Wash Station" connector on the Control-*xt* board, as shown below in Figure 11.

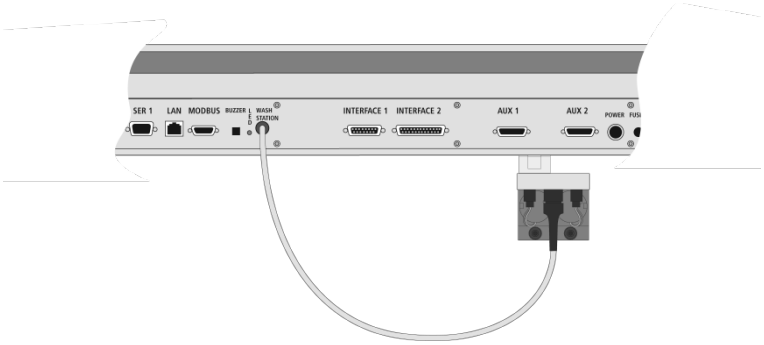

Figure 11. Electrical Connection from the DLW Pump Holder to the PAL-*xt* Board.

2.4. Installing the DLW Wash Station

The DLW Wash Station can be connected anywhere along the X-axis; however, it is strongly recommended to install it as close as possible to the Injection Valve. The travel path of the Injection Unit from the valve to the Wash Station should be as short as possible to keep the cycle time as brief as possible.
Figure 12 shows two possible configurations for installing DLW modules, favoring a short connection from the injection valve to the other HPLC system components on the one hand, and always having the DLW Wash Station close to the injection valve on the other. It is important that the DLW Pump Module, with the connected DLW tubing from the kit, always be attached on the far left side of the X-axis.
Such a configuration also has the advantage that the waste tube from the injection valve can be connected to the front "Waste Inlet" of the Wash Station module. This waste tube is part of the DLW Option Kit.

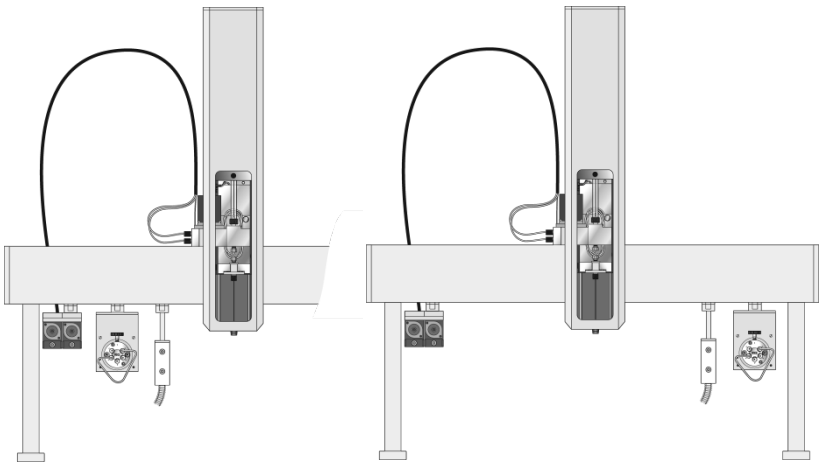

Figure 12. Mounting the DLW Option Modules in combination with the Injection Valve.

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1. Locate the DLW Wash Station module.
2. Attach the holder to the X-axis at selected position as shown above.
3. Connect the Waste Tube from the Injection Valve to the DLW Wash Station.
4. Connect the Waste Tube to the Wash Station Waste Adapter.

Replace the dummy plug(s) on the front if used to connect the waste line coming from the injection valve. This sequence of waste lines simplifies the system, avoiding several waste lines running to the waste container.

note

The other two dummy plugs underneath the waste block are used for the DLW option.
The DLW tubing from the DLW Pump outlet could be connected at these wash station inlet ports. The functionality of the Fast Wash Station would then be configured using this order of connections.

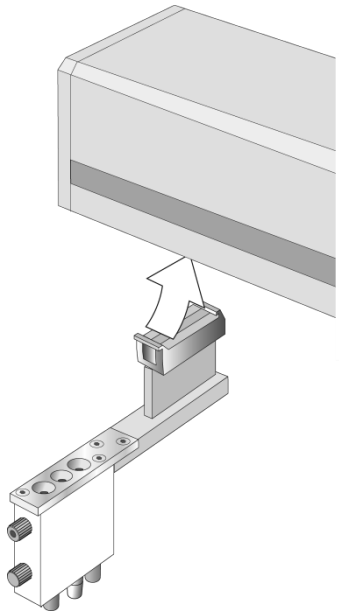

Figure 13. Attaching the DLW Wash Station to the X-Axis

note

The waste tube must be positioned below the injection valve.
Make sure that the waste liquid flows into the waste container without any restriction. The waste tube must always be placed above the liquid level in the waste container.

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2.5. Installing the DLW Syringe Holder Assembly

note

Before a syringe is inserted, the position "Change Syr" should first be verified. The position for "Change Syringe" should not occur above an object, for example, where the needle could collide with a vial.

Path:
Menu/Utilitites/Syringe/function key "F3" "Change Pos".

Move the PAL Injection Unit to a location where no collision can occur with the Z-axis by selecting the X- and Y- axes appropriately. The value for the Z-axis is given as a default and a change of this position is not necessary in standard operation.

This precaution helps avoid needle damage during routine operation. Nevertheless, the description below on how to install the DLW Syringe Assembly recommends switching off the unit and inserting the assembly without using the PAL command "Change Syringe".

The DLW Syringe Holder differs from standard liquid syringe holders because the DLW manifold is attached to the syringe holder. The DLW Actuator/Solenoid and the Holding Loop connecting the syringe inlet (bottom) and Syringe Needle are attached to the DLW manifold.
All of these parts are preinstalled at the factory. See Figure 14 below.

DLW Syringe Holder Assembly

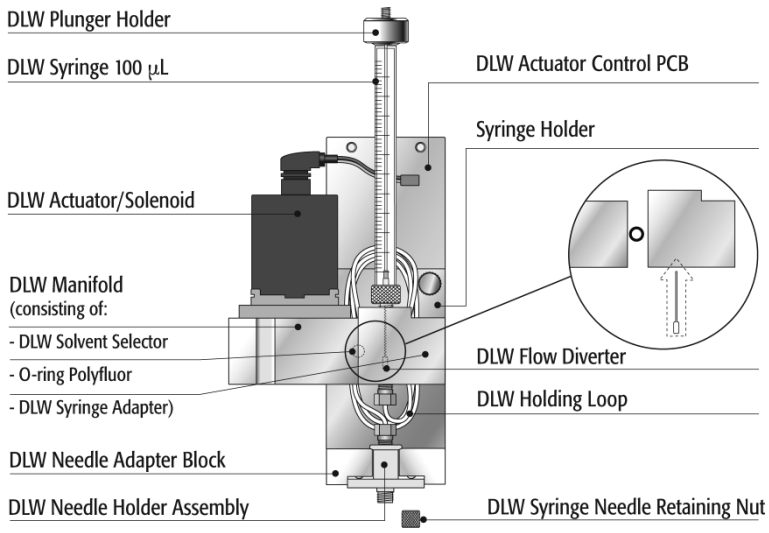

Figure 14. Front view of the DLW Syringe Holder assembly.

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2.5.1. Preparing and Installing the DLW Syringe

note

*The description on how to correctly install the Plunger Holder is given for field upgrade situations or when a plunger is replaced. Newly-shipped PAL **-xt** Systems which include the PAL DLW Option contains a preconfigured syringe with the Plunger Holder installed.*

1. Prepare the DLW Syringe by inserting the DLW Plunger Holder.
   First, manually move the plunger down to the stop position; release a slight amount of pressure from the plunger tip by pulling a fraction of a mm backwards. Install the DLW Plunger Holder and tighten the Allen screw (Allen Key #6) firmly.

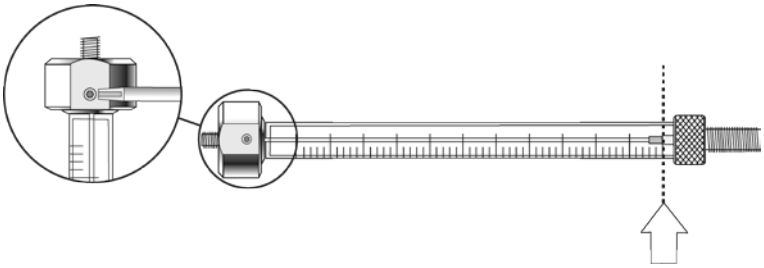

Figure15. Inserting DLW Plunger Holder on DLW Syringe

note

It is critical that syringes are primed before beginning sample preparation. Prime every liquid syringe manually before inserting into the PAL system.

2. Screw the prepared DLW syringe into the holder.
   Hold the syringe at the lower metal mount while tightening the syringe.

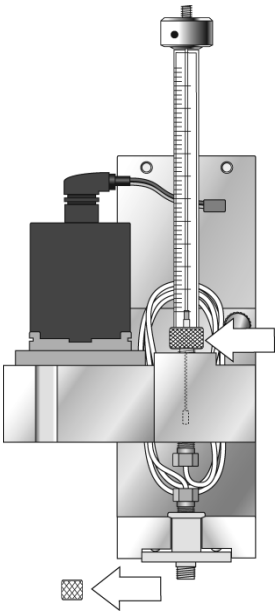

Figure 16. Inserting the DLW Syringe into the Holder.

note

Holding the glass barrel while tightening can damage the seal where the glass meets the metal.

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note *Do not mount the syringe needle at this stage.*

2.5.3. Connecting the DLW Tubing to the Syringe Holder

note *It is recommended to connect the DLW Tubing from the kit to the Syringe Holder while the assembly is not yet inserted in the X-axis.*

note *The wetted parts of the DLW Actuator/Solenoid are composed of PEEK for the body and FFKM (Simriz) for the seal material.*
PEEK exhibits excellent resistance to most commonly-used chemicals. However, the following solvents are not recommended for use with PEEK: DMSO, THF, methylene chloride (dichloromethane), nitric acid, sulfuric acid. For more details, see the compatibility tables provided by the manufacturer of PEEK materials or components.

1. As described under point 2.3 “Installing Pump Module”, the end of the the DLW kit sleeved tubing with the short tubes has to be connected to the DLW Syringe Holder Assembly.

2. Use the groove in the block at the back side of the DLW Manifold to orient the guide wire. Use the M3x5 screw provided and the corresponding serrated lock washer to connect.

3. Test if the wire tension is enough to keep the tubing in an upright position but low enough to move the syringe holder manually along the X-axis.

note *The design of the DLW Manifold and the concept of washing by active pumping and closing of the lines by the DLW Actuator does not require a predefined solvent line position, in theory.*
Problems could occur if solvent is refilled or exchanged while the tubing still contains solvent from the previous setup. Always keep the lines at the same position, and carefully prime the entire system to prevent unnecessary confusion. See also section G, “Operation”.

4. Connect solvent line 1 to the upper- and solvent line 2 to the lower port at the left side of the DLW Manifold.
   The order of the tube connections, upper and lower positions should be consistent. For certain applications, it is crucial not to mix types of solvents, e.g. biofluid sample solutions should not come in contact with highly concentrated organic solvents.

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2.5.3. Inserting the DLW Syringe Holder Assembly into the Injection Unit

note *It is recommended to insert the DLW Syringe Holder assembly when the PAL System is powered off.*

1. Move the PAL Injection Unit aside manually to allow free movement of the syringe slider. Lower the syringe slider as shown in Figure 17 in order to gain space for installing the DLW Syringe assembly.
   Match the magnetic pins of the syringe holder using the counter positions on the syringe slider.

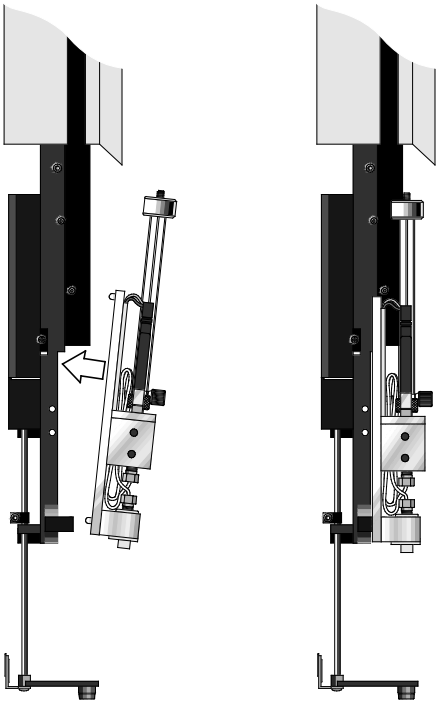

Figure 17. Inserting the DLW Syringe Holder Assembly into the Injection Unit.

2. Press the syringe holder firmly against the Z-axis slider to ensure that the holder clicks into place.

note *Do not press against the DLW Actuator/Solenoid. Its mounting to the DLW Manifold is fragile.*

3. Tighten the knurled screw to fix the Holder Assembly to the syringe slider (see Figure 18).

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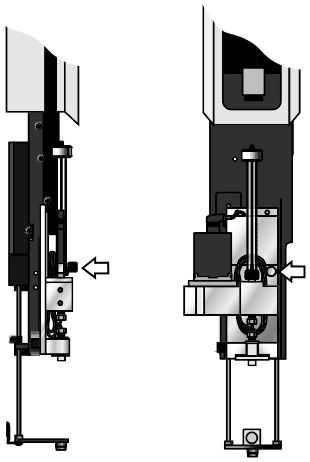

Figure 18. Fixing the Syringe Holder to the Syringe Slider.

4. Move the plunger up (plunger holder) until the thread of the screw catches the thread of plunger bushing. Tighten the screw to fix the plunger holder (see Figure 19).

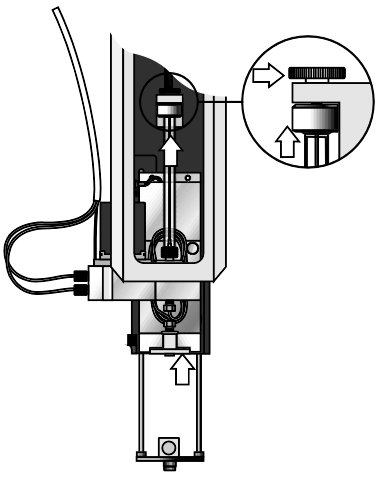

Figure 19. Connecting the DLW Syringe Plunger Holder.

5. Tighten the holding screw to secure the syringe holder position.

6. Insert the syringe needle tip into the Z-axis lower needle guide, then move the needle back up to the “DLW Needle Holder Assembly” and tighten firmly.

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note

The removable needle must be fixed to the syringe in a leak-tight manner. A Teflon seal is provided. When installing the seal, first ensure that the hole is open, and that no fuzzy (lint) polymer material which could clog the needle is in the path. When slipping the Teflon seal over the needle, pay attention to the sharp edges of the needle which could cut into the polymer. Again, this could clog the needle.

7. Move the lower needle guide carefully up and down to make sure that the needle tip does not catch on the guide.

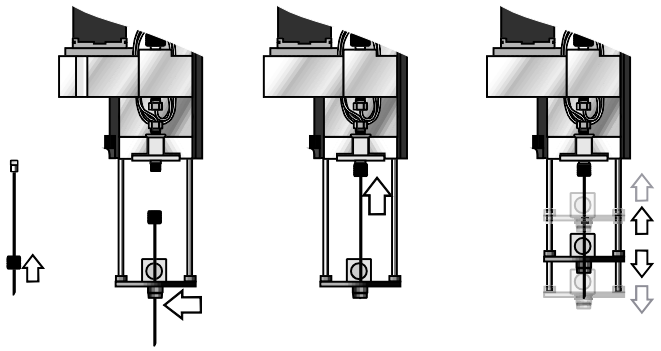

Figure 20. Inserting the DLW Syringe Needle.

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2.6. Installing the Valve Needle Guide Assembly

For a field upgrade, when first installing a DLW option, the standard valve needle guide can continue to be used. If the current Wash Station is the "Active Wash" type, replace the Needle Guide with the standard Needle Guide provided.

In all cases, unscrew the existing Needle Guide, remove the old needle seal inside the valve port and insert a new seal.

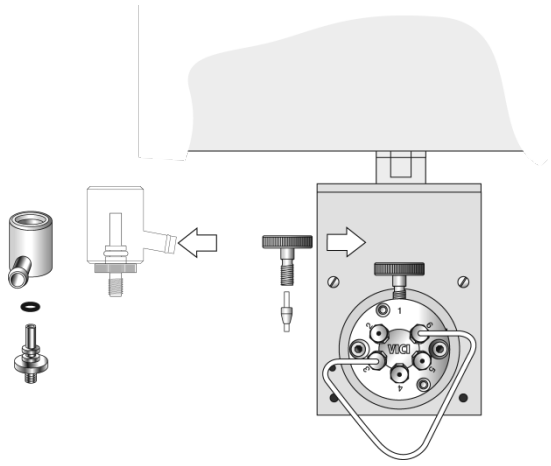

Figure 21. Installing the standard Valve Needle Guide.

2.7. Injection Valve Plumbing and Connection to the DLW Wash Station

1. Connect the injection valve Waste Line from port 2 (example standard valve Cheminert type), as shown in Figure 21, to the front Waste Port of the Wash Station.

note

If two injection valves are used in the PAL configuration, the second waste line can be connected to the lower waste port in front of the DLW Wash Station. If only one connector is used, apply a dummy plug to the second port on the Wash Station.

2. Place the Waste Tube from the DLW Wash Station into the waste container.

note

The waste container must be positioned below the injection valve. Make sure that the waste liquid flows into the waste container without restriction.

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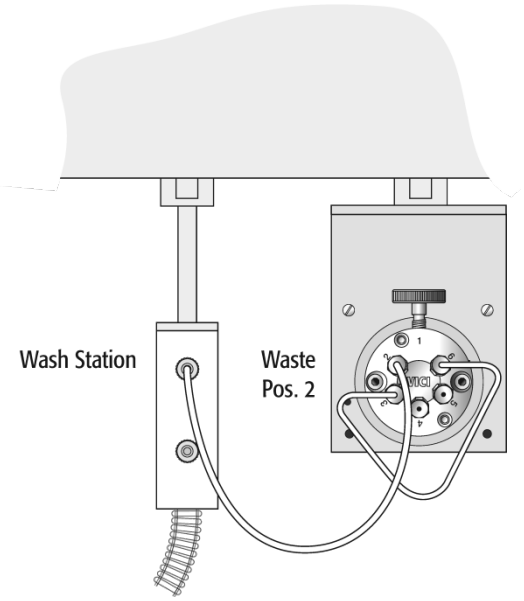

Figure 22. Solvent Line Plumbing on Cheminert Valve Types.

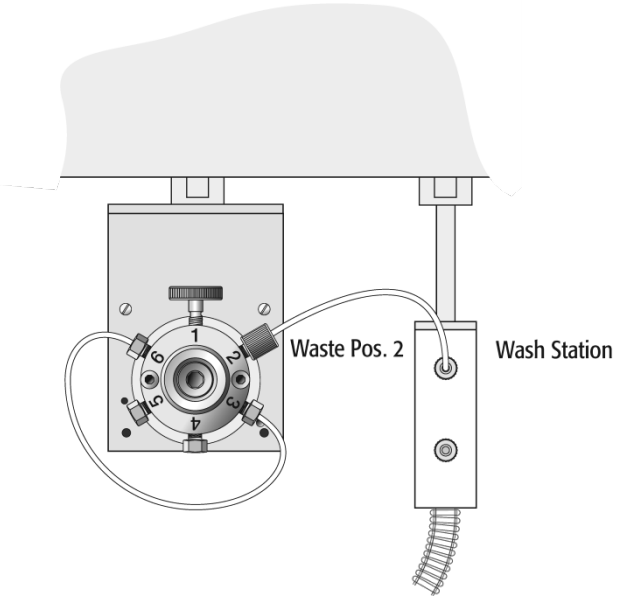

Figure 23. Solvent Line plumbing on Valco W-type Valves.

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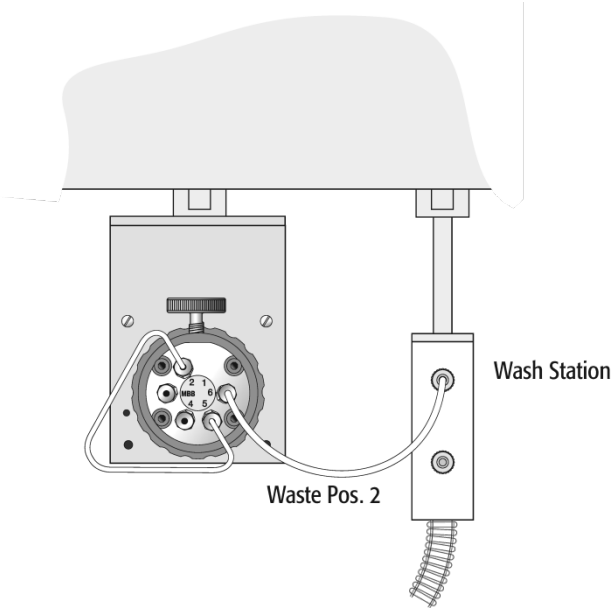

Figure 24. Solvent Line plumbing on Rheodyne Valve PD Series.

note The Agilent-specific Rheodyne Valve with Agilent PNo. 5067-412 is connected in the same manner to the HPLC system as shown in the corresponding HTS or HTC PAL User Manual for the Rheodyne type "PD7991". For details, see the specific valve description.

2.8. Electrical Connections for the Single Injection Valve Setup

note *Always switch the PAL power supply OFF before connecting or disconnecting the PAL DLW cable or any other PAL accessory cables!*

The electrical connection of the DLW option is identical to that of the PAL HTS-*xt* and the HTC-*xt* models. The same board is used for both models.

The schematic in Figure 25 shows an installation using the valve drive connected to the "AUX" interface. If a serial or multiposition valve drive is used, see schematics for 2- or 4-injection valve combinations (Figures 26 and 27).

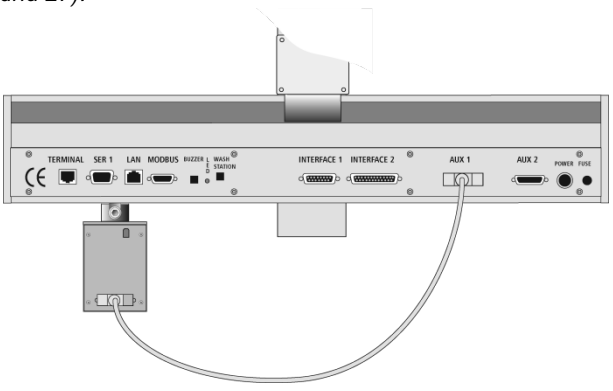

Figure 25. Electrical Connection, DLW Option on a PAL-*xt*

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2.9. DLW Option Installation in Combination with 2- or 4- Injection Valves

2.9.1. General System Overview

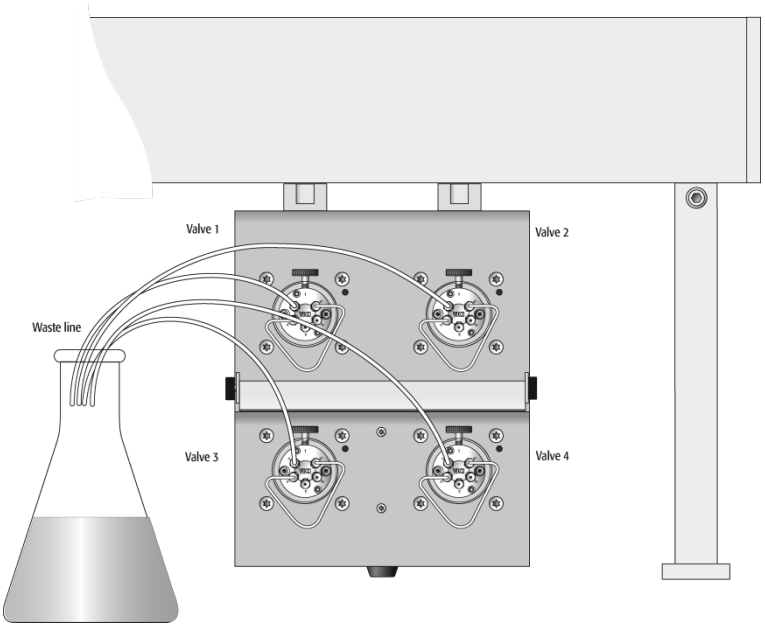

Figure 26. DLW Option in Combination with 4-Injection Valves.

The DLW Option can be combined with PAL Systems using a setup of 2- or 4-injection valves. See Figure 26. The concept of the DLW option is that the crucial parts of the wash station are attached to the injection unit. The sample is taken up into the Holding Loop, the syringe is actually the prepared reservoir for the following wash step after the loop filling and injection steps.
Following this concept allows for more than one injection valve in the PAL configuration. No special setups or additional modules are required to inject into and clean one or more valves connected to the PAL System. As described above, the crucial DLW parts are attached to the injection unit at any available position.

2.9.2. Electrical Connections for 2- or 4-Injection Valves

Connect the valve drives as shown below in Figure 27.

Note that the DLW option can only be used in combination with PAL-*xt* systems. Therefore, connect the first cable from the PAL Interface "MODBUS" to the serial valve drive connector "A". Each drive is connected with daisy-chain cabling.
The last serial drive connector "B" remains open or unused. Electrical current and interface logic is provided by the interface "MODBUS".

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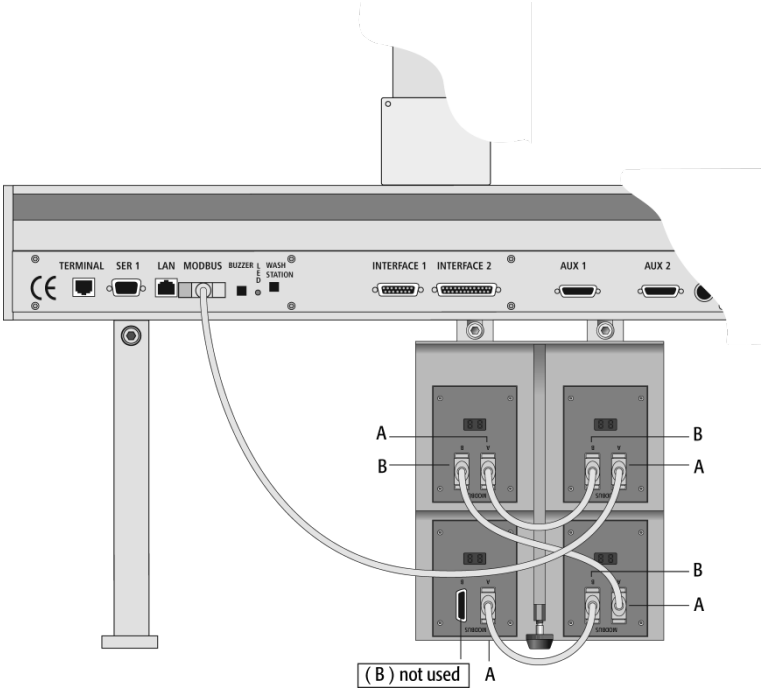

Figure 27. Electrical Connections to 4-Injection Valves, Daisy-Chaining with PAL-*xt* System.

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F. PAL Firmware and PAL Firmware Object Installation for the DLW Option

1. PAL Firmware

The operation of the DLW option is only possible with the PAL-*xt* System, which requires the APR Control-*xt* board and PAL Firmware version 4.1.X.

The DLW option is NOT compatible with a PAL System operating with PAL Firmware version 3.0.X or lower.

The DLW option can be controlled with either a Cycle Composer macro or with any data system that controls the PAL, using Cycle Composer macros or PAL ICC cycles (e.g. Analyst, ChemStation, Empower, EZChrom, MassLynx, Xcalibur).

2. Loading the PAL DLW Object

If the version of the PAL Object Manager List installed on the computer is not "PAL-*xt* Object Lists Rev. B", then copy the provided folder "Wash Station Option" (see Fig. 28.) from the CD-ROM to the Object Lists folder which has been installed with the Object Manager. This software is usually installed in the following path:

C:\Program Files\PAL\Object Manager\Object Lists

Using PAL-*xt* Object List Rev. B or higher, the DLW Option object will be integrated into the list in the "Wash Stations" folder.
The following Object List is then available:

PAL DLW: DLW Option Firmware Object List

1. Open the Object Manager and verify the revision version number of the Object Lists.
   If PAL-*xt* Object Lists Revision level "B" (or higher) is available, continue as described below.
   If a "PAL Object Lists" version is used (e.g. Rev. K or L), copy the provided DLW Object List into the Object Manager folder as described above.

2. Select the "PAL DLW" Object list and click "Send selected Object Lists to PAL".

3. Close the Object Manager.

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The DLW Object list contains the following PAL Firmware Objects which are loaded with a single command as described above:

Object Class	Object Name
Motor Drive	MPlgMed
Heater	HDLW
Syringe	100ulDLW
Wash Station	Wash1
	Wash 2
Injector	Waste
PWR Event	Pwr-Out1
	Pwr-Out2

Table 2. DLW Object List.

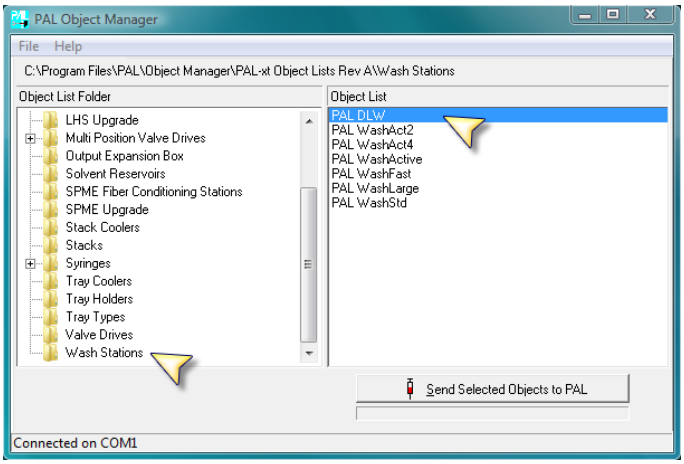

Figure 28. PAL Object Manager; choosing Object List Folder "Wash Stations".

note

For more details on the PAL Object Manger software, see the Addendum to the PAL User Manual "PAL Object Manager Software".

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3. Wash Station Reference Points

3.1. Wash1/Wash2 Reference Points

For the DLW Wash Station Option, the reference positions for "Wash1" and "Wash2" are the two holes on top of the Wash Station assembly. See Figure 29.
The lower needle guide should be centered on these holes with the bottom of the lower needle guide lightly touching the surface of the Wash Station assembly.

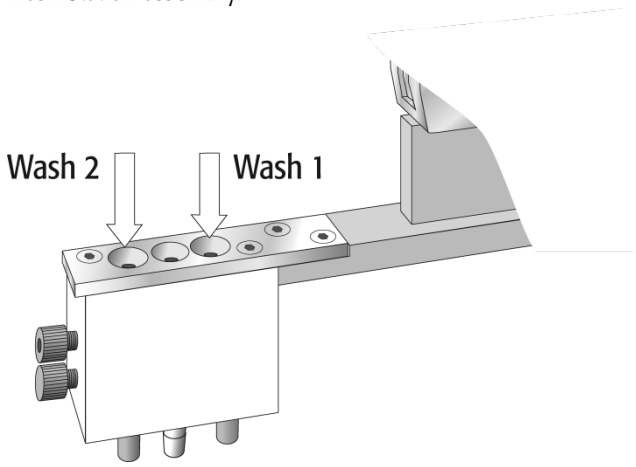

Figure 29. DLW Wash Station Wash1/Wash2 Reference Points.

1. Press "HOME" on the handheld controller and select the sequence:

   Menu/Setup/Objects/Wash Stations/Wash1.

2. Enter Wash1 and adjust the X-, Y-, Z-positions until the lower needle guide of the Injection Unit centers on top of the Wash station assembly. Fine tune the Z-position until the bottom of the lower needle guide is flush with the top surface.

3. Repeat steps 1 – 3 for Wash2 using the following path:

   Menu/Setup/Objects/Wash Stations/Wash2

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3.2. Waste Reference Point

Injectors (Waste)

note

The Waste position is defined as an "Injector" by the PAL software. It is classified in the Object category "Injectors".

The reference position for the **Waste Port –** a hole (slightly larger than the needle guide) – has been placed in the middle positions of "Wash1" and "Wash2" (see Fig. 30). The lower needle guide should be centered on this hole with the bottom of the lower needle guide lightly touching the surface of the Wash Station assembly.

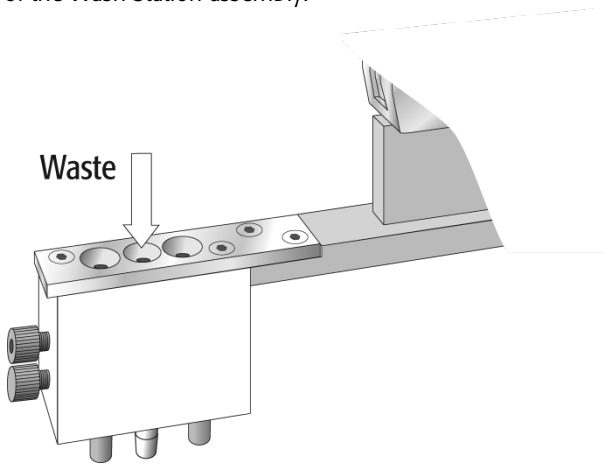

Figure 30. Waste Reference Position.

1. Using the hand-held controller, select the sequence:

   Menu/Setup/Objects/Injectors/Waste 1.

2. Enter Waste and adjust the X-, Y-, Z-positions until the lower needle guide of the Injection Unit centers on top of the Wash Station assembly. Fine tune the Z-Position until the bottom of the lower needle guide is flush with the top surface.

3. Press "F1" to verify the position and "F4" for "Home".

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4. Injection Valve Reference Points

For an **LC Valve**, the reference position is the valve needle guide fitting mounted on the top valve port (see Fig. 31).

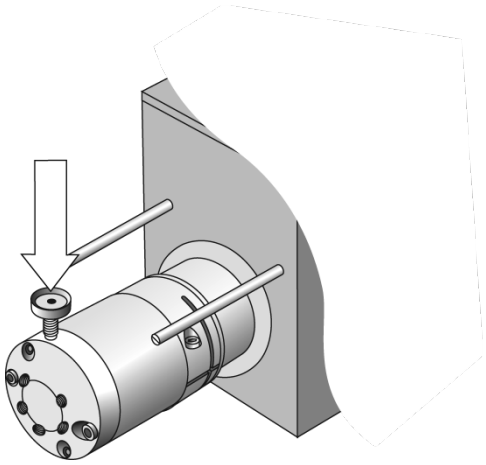

Figure 31. LC-Valve Reference Point.

The lower needle guide of the Injection Unit is centered on the valve needle guide fitting. Adjust the Z-Position so that the bottom of the lower needle guide just touches the surface of the valve needle guide fitting. **Then reduce this value by 2.0 mm.** See Figure 32.

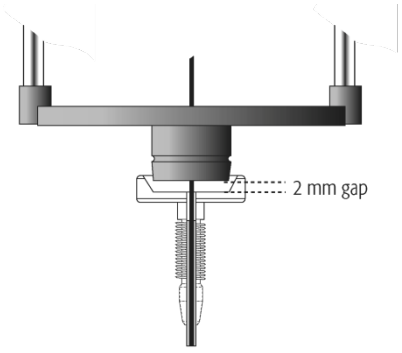

Figure 32. Positioning the Z-Axis Needle Guide on the Valve Needle Guide.

note *To adjust the Valve Needle penetration value, see point 5 below, "Defining the Valve Needle Penetration Value".*

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5. Defining the Valve Penetration Value

note

Before performing the following steps, make sure that Object positions X, Y, and Z for LC Vlv1 are properly defined and that the complete DLW syringe assembly is inserted into the injection unit.

Complete the following steps to define the Valve Needle Penetration Depth:

1. Using the hand-held controller, select the sequence:

   Menu/Setup/Objects/Injectors/LC Vlv1.

2. Press the F1 button "Check Pos". The Injection Unit moves to the previously defined LC Vlv1position.

3. Highlight the item “Needle Penetr” with the cursor bars by pressing ENTER.

4. Slowly rotate the outer knob to adjust the needle penetration depth. The syringe moves down stepwise into the injection port.

5. When the syringe needle tip enters the valve needle guide, slow down the Z-movement again. Always observe the syringe needle during this adjustment step.

6. Move down stepwise until the bottom of the cross bar of the DLW Needle Holder Assembly is flush with the lower line of the “DLW Needle Adapter Block”. See Figure 33 below.

7. Press ENTER to save the needle penetration depth value.

8. Verify the defined Needle Penetration Depth value by repeating steps 3 to 6.

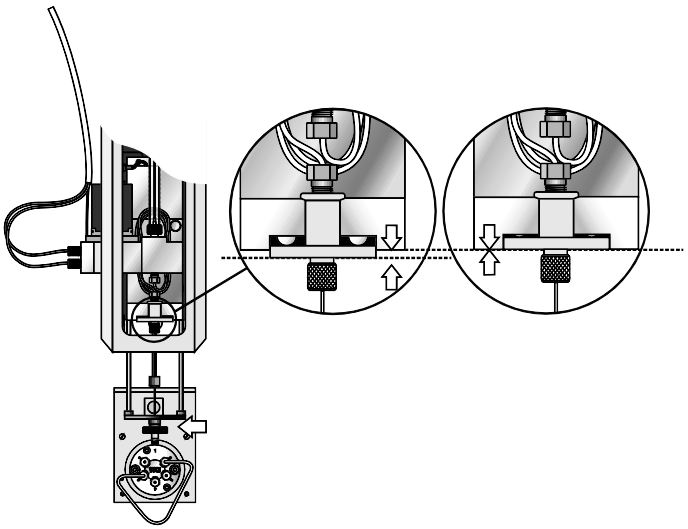

Figure 33. Teaching the Needle Penetration Depth Value

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note

If a "click" sound occurs, this means the ball-spring loaded "Needle Holder" is completely compressed. STOP the Z-axis downwards movement immediately. Rotate the outer knob stepwise in the opposite direction until the gap in the spring-loaded "Needle Holder" is approximately 1 mm, or the bottom of the "Holder Assembly" is flush with the lower surface of the "Needle Adapter Block", as in Figure 30. Repeat the needle penetration test and verify the needle penetration depth.

An example of the correct and incorrect positioning of the syringe needle tip in the valve inlet port is shown in Figure 34.

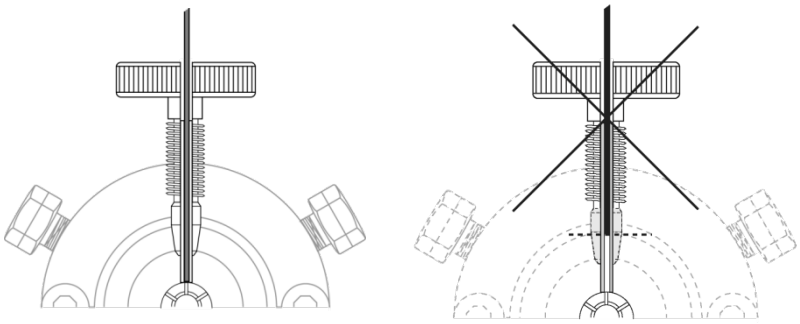

Figure 34. Correct and incorrect Valve Needle penetration depth.

The ball-spring loaded "Needle Holder" presses the square cut needle tip firmly against the bottom of the valve body. This ensures a constant seal during the injection process.

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G. Operating Instructions

1. Priming the Solvent Lines

note

For trouble-free DLW operation, make sure the two solvent lines are free of air bubbles at all times. If the solvent lines are being connected for the first time or during a solvent change, it is necessary to prime the solvent lines properly until air bubbles are no longer visible. Solvent degassing and filtering is recommended.

The priming of the PAL DLW Option is conveniently done directly from the local terminal. There are several tasks which can be executed from the 'Utilities' section. It is recommended to use the function of the Wash station first, followed by 'Clean Syringe'.

Please follow this description step-by-step to prime the solvent lines and the entire DLW system. Solvent degassing is recommended.
Important is to observe the sequence of steps to ensure that Solvent 1 will be the last solvent in the DLW System. Good practice is to have the wash solvent with lower chromatographic strength (elution power) defined as 'Wash Solvent 1' and 'Wash Solvent 2' logically consistent as the solvent with a stronger elution power.

1.1 Priming with Utilities Function 'Wash Station'

Prime first the PAL DLW System with the Utilities function 'Wash Station':

Select Menu | Utilities | Wash Station | select Wash 2 | Function Key "F3" 'Move to Wash' | Function Key "F2"Activate Valve |.

Observe the green LED as an indicator that the DLW Actuator is activated. Prime with Solvent 2 until no further air bubbles are observed.

Repeat the steps to prime with Solvent 1, by activating Wash 1.

1.2. Priming with Utlitities Function 'Syringe'

As second priming step for the PAL DLW System is the Utilities function 'Syringe' recommended. This function allows controlling the plunger movement. It is important that the priming through 'Wash Station' is completed, the solvent lines are free of air bubbles.

Select Menu | Utilities | Syringe | Function Key "F2" 'Cln Syr' | select Wash 2
select the desired 'Clean Count' (recommended a minimum of 3 counts)

Observe the green LED as an indicator that the DLW Actuator is activated and the smooth plunger movement. Prime with Solvent 2 until no further air bubbles are observed.

Repeat the steps to prime with Solvent 1, by activating Wash 1.

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1.3. Other Priming functions or Troubleshooting

Another priming function is available in section Utilities | Injector.
Select 'Clean Injector and follow the dialog with similar logic as described above.

In case any problems are observed, follow the instructions given in Section H, Troubleshooting. Dedicated tests to verify the DLW functionality are described.

2. Location of Solvent and Waste Bottles

The DLW Option contains self-priming membrane pumps. The solvent bottles can be placed either in the Fast Wash Station Holder or on the lab bench.

The Waste bottle must be placed ≥ 30 cm below the injection valve. Make sure that the waste liquid can flow into the waste bottle without restriction. The waste tubing must be placed above the level of the liquid. Ideally, the tube should be fixed at the neck of the waste bottle.

note

Use good lab practice to avoid contaminating the wash solvents and bottles. Avoid the growth of biological contaminants in pure water by either replacing it regularly or adding a small amount of organic solvent, such as methanol or acetonitrile. Certain buffer solutions can decompose at room temperature when exposed to light. Filtering the wash solvents before filling the bottles, especially if salt buffers are used, is mandatory to avoid clogging of the solvent paths.

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3. Functionality of the DLW Option

3.1. DLW Pumps

From the control point of view, the DLW pumps respond in the same manner as the Fast Wash Station. The pumps are activated by "Power-out Signals". The electric current setting for the DLW is different; this means that the corresponding PAL Firmware Objects have to be loaded for each DLW wash station type. See Section F, point 2 "Loading PAL DLW Objects" for further details.

The wetted parts in the pump are made of the following materials:

- Membrane: Kalrez (FFPM)
- Body, Valves: Ryton PPS

The pumps are self priming with a suction lift of up to 3 m water column.

3.2. DLW Actuator/Solenoid

The "DLW Actuator/Solenoid" separates and completely shuts off the lines in the direction of the syringe (sample loading) and the wash solvent. After opening the DLW Actuator/Solenoid for the wash solvent lines, the desired wash solvent can be pumped into the system by activating the corresponding DLW pump.
The schematic in Figures 35 and 36 illustrates this function.

The wetted parts in the DLW Actuator Solenoid are made of the following materials:

- Solenoid body: PEEK
- Seal material: FFKM (Simriz)

note *PEEK exhibits excellent resistance to most commonly-used chemicals. However, the following solvents are not recommended for use with PEEK: DMSO, THF, methylene chloride (dichloromethane), nitric acid, sulfuric acid. For more details, see the compatibility tables provided by the manufacturer of PEEK materials or components.*

note *A green LED is activated when current is applied from the actuator control PCB to the actuator/solenoid.*
This activation does not mean that the solenoid will open or close. In cases of defects, such as a defective or clogged Actuator/Solenoid, would the LED lighten up but the solvent path is not opened.

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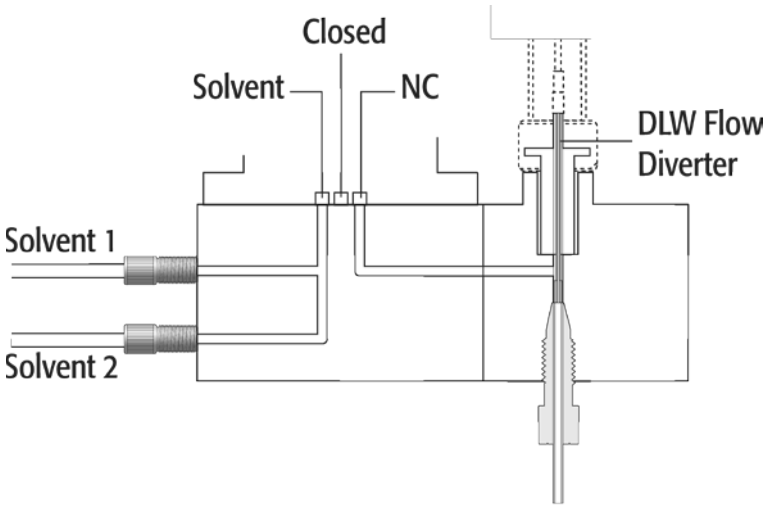

Figure 35. DLW Manifold and Actuator/Solenoid.

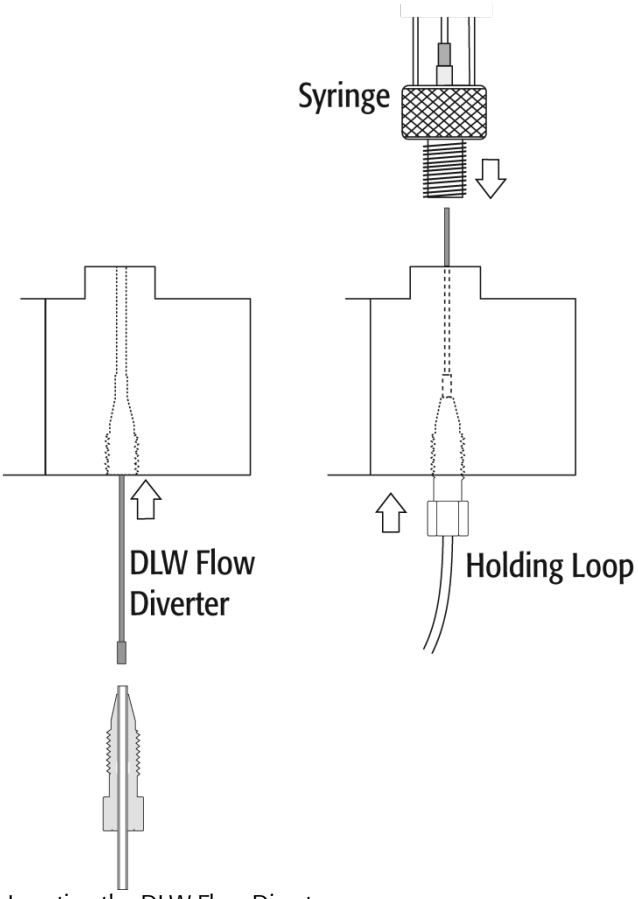

Figure 36. Inserting the DLW Flow Diverter.

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4. Installing Cycle Composer Macros or ICC Cycles used for the DLW Option

The PAL DLW Option can only be operated in combination with PAL control software, such as Cycle Composer or the Cycle Editor, for ICC interpretation in another data handling system software.

For software control, two macros or two cycles are provided.
The first macro is used for "Standard" speed with optimized washing possibilities, and the second for a "Fast" cycle with optimized throughput and less focus on carry-over. See Table 2 below.
The two macros (or cycles) are explained in detail; see Tables 3 – 5 below.

Macro Name	Macro Description
LC-Inj DLW Standard	Standard injection cycle using all possibilities of the DLW Option. The injection valve inlet port and the needle are washed with both wash solvents (inside and out). An extra "Stator Wash" can be added for intensive washing of the injection valve (valve toggle).
LC-Inj DLW Fast	The macro is tuned for speed and high-throughput application. The difference with regard to the Standard DLW macro is that some steps are left out to shorten the cycle time. Details are explained below.

Table 3. The DLW Macro definitions.

4.1. Installing the Cycle Composer Macros or ICC Cycles

The PAL DLW Option is shipped with a CD-ROM 'PAL System' containing various cycles (macros) for the DLW option.
Copy these macros to your Cycle Composer Method folder or the corresponding folder for applications within the integrated system.
Navigate in Explorer to the Cycle Composer folder. This is usually installed in the following path:

C:\Program Files\PAL\Cycle Composer

It is advisable to create a separate Method Folder for the DLW Option.
Copy the folder "DLW Option" from the CD to the Cycle Composer folder.
In Cycle Composer, use the menu "File/Options/Choose Method Folder…" and select the DLW Option folder as the Method folder.

If you wish to add the DLW Option macros to an existing Method folder, just copy the macros (*.pma) and the methods (*.pme) files from the "DLW Option" folder on the CD-ROM to the appropriate folder.

If the PAL System is integrated with a data system software that controls the PAL using the Cycle Editor for PAL ICC interpretation (e.g. Analyst, ChemStation, Empower, EZChrom, MassLynx, Xcalibur), an ICC "Cycle" is used and not the Cycle Composer "Macro", the cycle extension is "*.cyx". Follow the instructions as described above. The folder for the cycle is usually located within the data system application folder.

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note *A Cycle Composer macro can be converted to a Cycle file format (extension "*.cyx") using the Cycle Editor software. Conversion is possible starting with Cycle Editor Version 1.4.0.4.*

note *The macros provided are written for standard injection valve drives, which are controlled and activated through the "AUX" interface.*

4.2. General Considerations

The duration of the wash steps has to be established for each configuration and application. The viscosity and surface tension of the individual wash solvents and the backpressure of the system are factors to consider.
Be aware that a higher backpressure builds up if the valve bore size or the installed loop internal diameter are lower (standard valve bore 0.25 mm).
The standard loop internal diameter (ID) for loops provided by CTC Analytics with a volume of 5, 10 and 20 µL is 0.25 mm. The loop with 2 µL content volume has an ID of 0.125 mm.
Keep the internal diameters of the tubing in line with the valve dimensions, loop ID and flow rate. For details see the poster "Tips & Hints for HPLC Technique".

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4.3. Standard DLW Injection Cycle

Macro Name: LC-Inj DLW Standard

Macro Description	Macro Variable
The PAL System waits for the Sync Signal "Ready" before the injection cycle is started.	*Remark:* *Sync Signal setting "Start"* *Fig.37*
The Injection Valve is brought to a defined position: "Standby".	Inject to Standby
The "Rear" air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed Fig. 38
The combined volumes of "Rear-", Sample List-", and "Front-Volume" are aspirated into the Holding Loop. The "Front" air segment is aspirated.	Front Volume Fig. 39 Rear Volume (SL.volume) Airgap Volume Filling Speed Pullup Delay Fig. 40
The Injection Unit moves to the DLW Wash station, position "Wash1". The needle is inserted (dipped) for 1 second to wash the outer needle surface. No plunger movement at this step.	Fig. 41
The Injection Unit moves to the specified Injection Valve. The "Front-" and "Airgap-Volume" are ejected.	Inject to Front Volume Airgap Volume Injection Speed Fig. 42
The PAL System waits for the signal from the data system.	"Wait for DS"
The Injection Valve is switched to position "Active". The system pauses according to the delay time "Pre Inject Delay". The loop is filled with the sample volume as specified in the Sample List. The Injection Valve is switched to position "Standby", the loop contents are injected. Timer 1 ("Delay Stator Wash" is started and a "start" signal to the HPLC system is sent.	Inject to Pre Inject Delay Fig. 43 (SL.volume) Injection Speed Post Inject Delay Timer 1 Fig. 44
The plunger of the DLW Syringe is pushed down to dispense the "Rear Sample" and "Air Segment" to Waste. The Holding Loop is still filled with "Wash Solvent 1".	(Syr. Eject Speed) Fig. 45
The DLW Actuator/Solenoid is activated to deliver "Wash Solvent 2" into the Holding Loop to clean the Injection Valve from Port 1 to Port 'Waste'. For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point. *Remark: The Atom "Rinse Inj" is new, available starting with FW 4.1.X.* *The DLW Actuator/Solenoid is activated, the Wash Solvent (pump), the "Needle Gap" and the "Rinse Time" are selectable.*	Wash2 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 2 Needle Gap is a parameter from "Rinse Inj" Atom. The variable in this macro is: Needle Gap Valve Clean". The function of this parameter is to lift the needle in the injection port to allow rinsing around the needle tip. The pressure of the spring-loaded balls in the DLW Syringe Holder Assembly is released by moving approx 3 mm up (default). This leaves a gap between the needle tip and the valve bottom of approx. 1 mm, to enable a flush at this contact point. Fig. 46
The Injection Unit is moved to the DLW Wash Station, position "Wash2". The needle is rinsed inside and out with "Wash Solvent 2".	Wash2 (Syr.Eject Speed) Post Clean Time Solvent 2 Fig. 47
The Injection Unit is moved back to the Injection Valve. The Inlet Port and engraving to waste Port is flushed with "Wash Solvent 1" to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1 Fig. 48

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The Injection Unit is moved back to the DLW Wash Station, position "Wash1", to flush the syringe needle inside and out with "Wash Solvent 1". This is a preparation step for next injection. Especially important for biofluid samples.	Wash1 Post Clean Time Solvent 1 Fig. 49
Cycle end for "LC-Inj DLW Standard" macro.	Fig. 50
An optional cleaning step is attached to the "DLW Standard" injection cycle: Stator Wash or Valve Toggle. A "Repeat-End" loop can be activated with the "Count".	Stator Wash Stator Wash count: 1 = Cleaning step active Stator Wash count: 0 = Cleaning step disabled
If "Stator Wash" is activated, the following steps will be executed.	
The Injection Unit is moved to the Injection Valve. From the last step above, the Holding Loop is filled with "Wash Solvent 1". Timer 1 is awaited to switch the valve (Toggle) into "Active" position (fill loop).	Inject to Delay Stator Wash (Active) Fig. 53
The DLW Actuator/Solenoid is activated to deliver "Wash Solvent 2" to the Holding Loop and into the valve system. The first solvent flush arriving at the valve is "Wash Solvent 1" followed by "Wash Solvent 2".	Inject to Wash2 Stator Wash Time Solvent 2 Fig. 54
The Wash Solvent is changed to "Wash Solvent 1".	Inject to Fig. 55 Wash1 Stator Wash Time Solvent 1 Fig. 56
The Injection Valve is switched back to the "Standby" position.	Inject to (Standby) Fig. 57

Table 4. "LC-Inj DLW Standard" Macro.

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4.4. Fast DLW Injection Cycle

The "Fast" injection cycle differs from the "Standard" cycle in three points:

- The needle is not dipped in the Wash station "Wash1" after sample pickup and before moving to the Injection Valve.
- The wash steps after injection are reduced to "Valve Clean" with Wash Solvent 1 and Wash Solvent 2. The DLW needle is flushed in the DLW Wash Station with "Wash Solvent 1" only.
- "Stator Wash" (Valve Toggle) is not available.

Macro Name: LC-Inj DLW Fast

Macro Description	Macro Variable
The PAL System waits for the Sync Signal "Ready" before the injection cycle is started.	*Remark:* *Sync Signal setting "Start"* *Fig. 58*
The Injection Valve is brought to a defined position: "Standby".	Inject to Standby
The "Rear" air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed Fig. 59
The combined volumes of "Rear-", Sample List-", and "Front-Volume" are aspirated into the Holding Loop. The "Front" air segment is aspirated.	Front Volume Fig. 60 Rear Volume (SL.volume) Airgap Volume Filling Speed Pullup Delay Fig. 61
The Injection Unit moves to the specified Injection Valve. The "Front-" and "Airgap-Volume" is ejected to "Waste".	Inject to Front Volume Airgap Volume Injection Speed Fig. 62
The PAL System waits for a signal from the data system.	"Wait for DS"
The Injection Valve is switched to position "Active". The system pauses according to the delay time "Pre Inject Delay". The loop is filled with the sample volume as specified in the sample List. The Injection Valve is switched to position "Standby", the loop contents are injected.	Inject to Pre Injection Delay (SL.volume) Fig. 63 Injection Speed Post Inject Delay Fig. 64
The plunger of the DLW Syringe is pushed down to dispense the "Rear Sample" and "Air Segment" to Waste. The Holding Loop is still filled with "Wash Solvent 1".	Injection Speed Fig. 65
The DLW Actuator/Solenoid is activated to deliver "Wash Solvent 2" into the Holding Loop to clean the Injection Valve from Port 1 to Port 'Waste'. For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point. *Remark: The Atom "Rinse Inj" is new, available starting with FW 4.1.X.* *The DLW Actuator/Solenoid is activated, the Wash Solvent (pump), the "Needle Gap" and the "Rinse Time" are selectable.*	Inject to Wash2 Needle Gap Valve Clean Valve Clean Time Solvent 2 Needle Gap is a parameter from "Rinse Inj" Atom. The variable in this macro is: Needle Gap Valve Clean". The function of this parameter is to lift the needle in the injection port to allow rinsing around the needle tip. The pressure of the spring-loaded balls in the DLW Syringe Holder Assembly is released by moving approx 3 mm up (default). This leaves a gap between the needle tip and the valve bottom of approx. 1 mm, to enable a flush at this contact point. Fig. 66

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"Wash Solvent 1" follows to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1	Fig. 67
The Injection Unit is moved back to the DLW Wash station, position "Wash1" to flush the syringe needle inside and out with "Wash Solvent 1". This is a preparation step for the next injection. Especially important for biofluid samples.	Wash1 Post Clean Time Solvent 1	Fig. 68
Cycle end for "LC-Inj DLW Fast" macro.		Fig. 69

Table 5. Macro LC-Inj DLW Fast

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5. PAL DLW Cycle Step-by-Step

5.1. Standard Cycle

Figures 37 to 50. Standard Injection Cycle Step-by-Step.

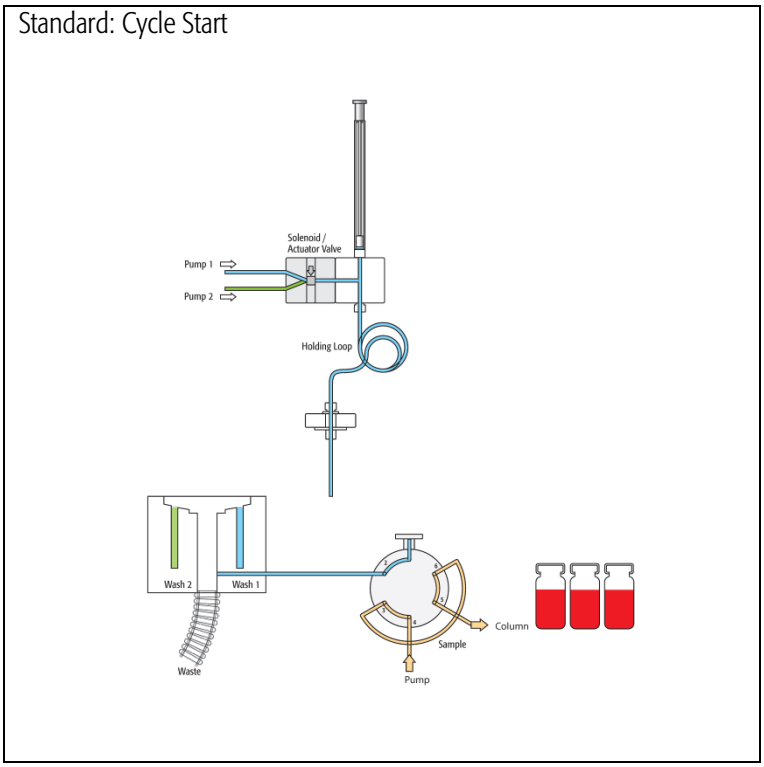

Figure 37.

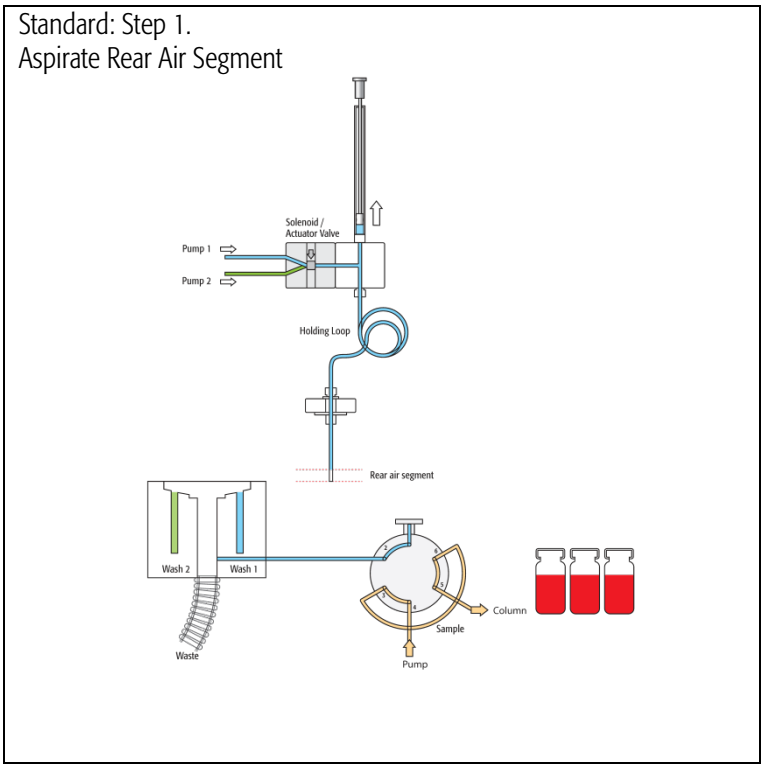

Figure 38.

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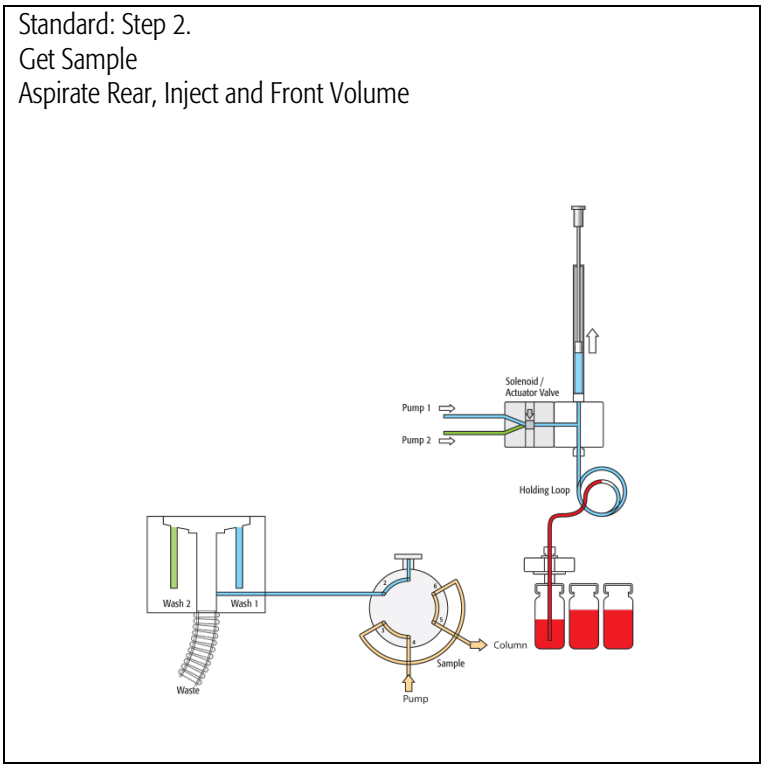

Figure 39.

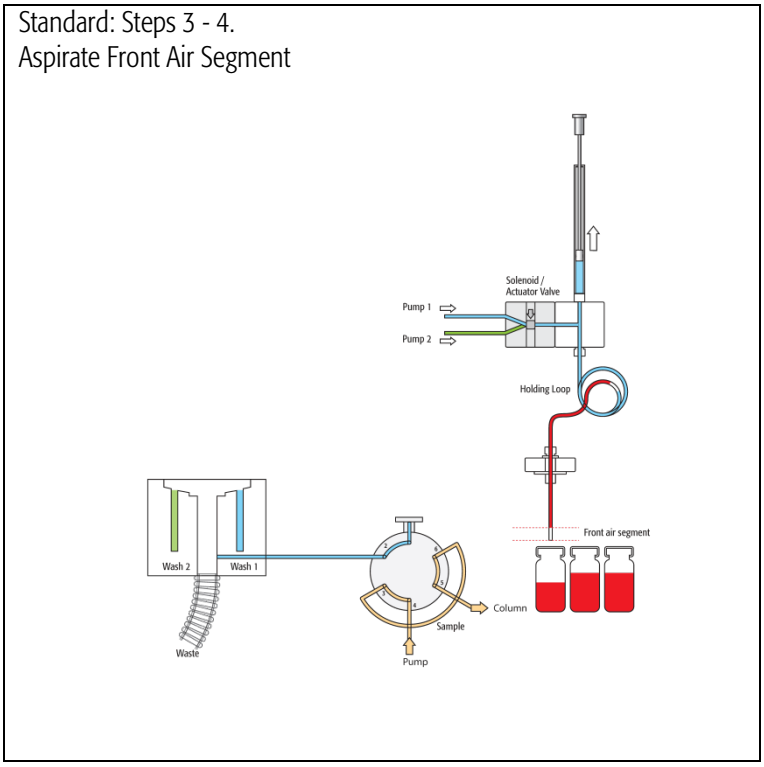

Figure 40.

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Standard: Steps 5 - 6.
Passive needle clean outside (dip) in wash position 1.

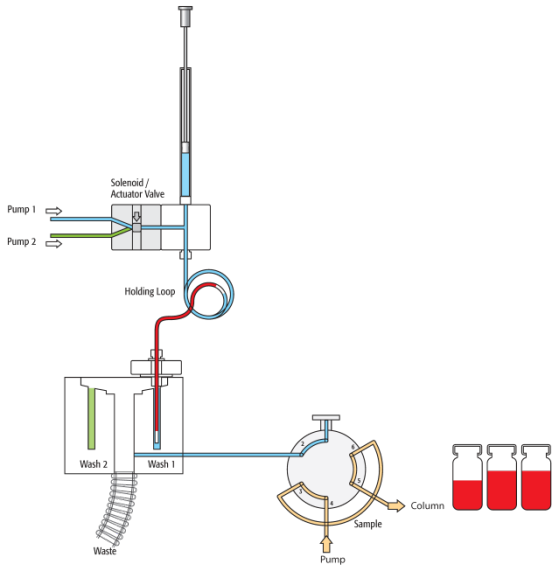

Figure 41.

Standard: Steps 7 - 8.
Dispense Front Air Segment and Front Sample Volume to Waste.

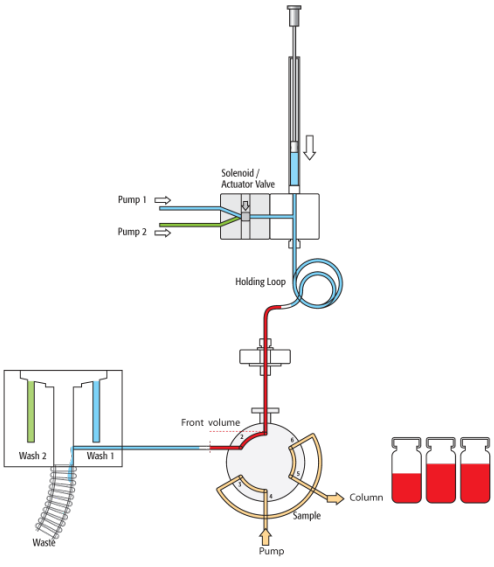

Figure 42.

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Standard: Steps 9 - 10.
Valve is switched to LOAD position, Loop is filled with “Inject Volume”.

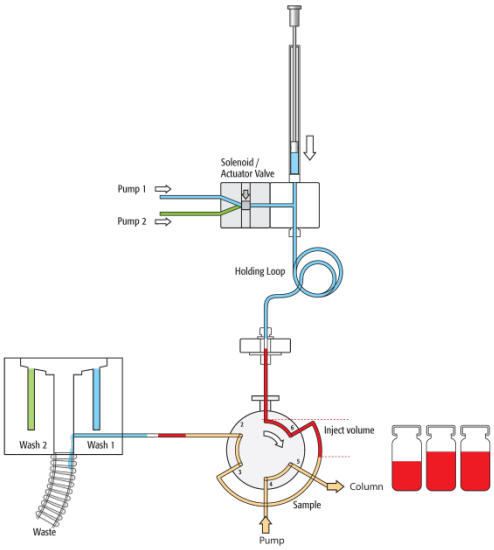

Figure 43.

Standard: Step 11.
Valve is switched to INJECT position, start chromatographic process.

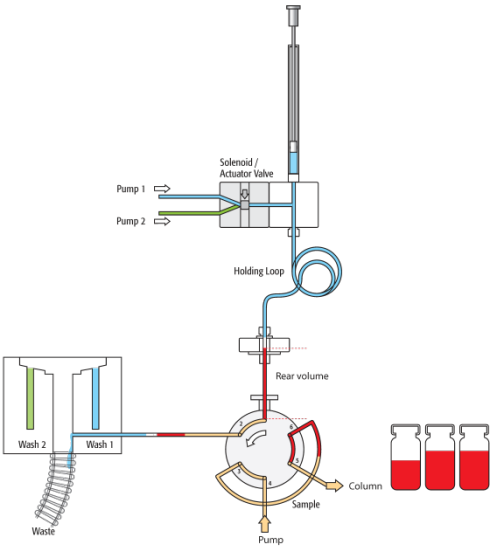

Figure 44.

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Standard: Step 12.
Rear Sample Volume and Air Segment are dispensed to Waste.

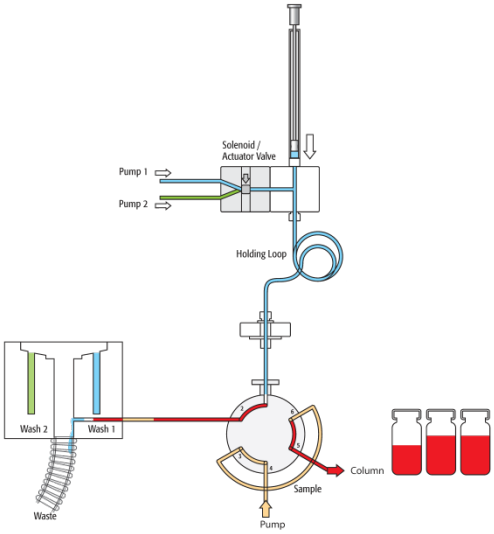

Figure 45.

Standard: Steps 13 - 14.
Valve Clean with Wash Solvent 2

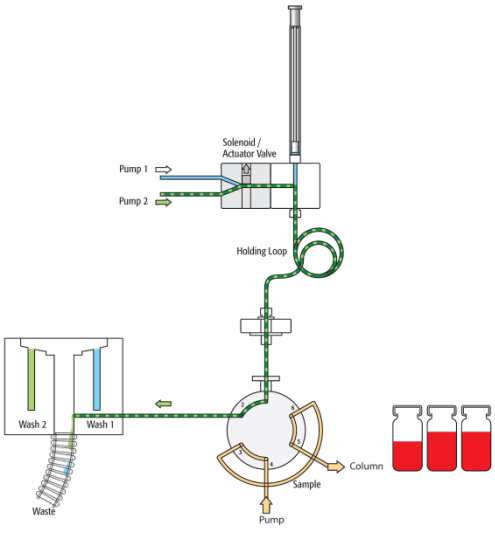

Figure 46.

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Standard: Steps 15 - 16.
Active Syringe Needle wash with Wash Solvent 2.

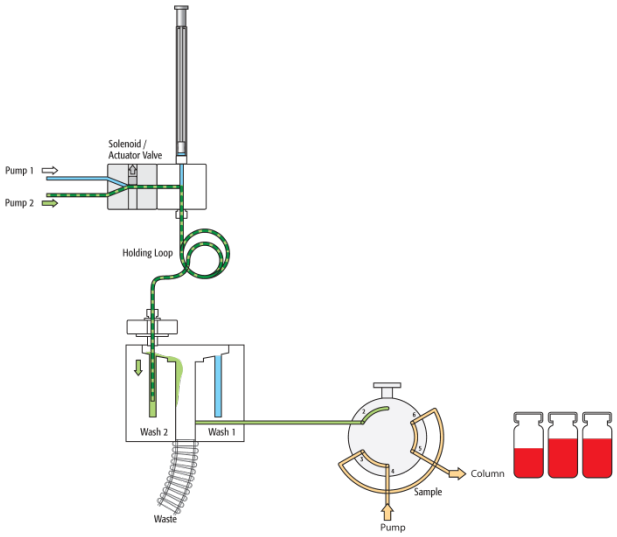

Figure 47.

Standard: Steps 17 - 18.
Valve Clean with Wash Solvent 1.

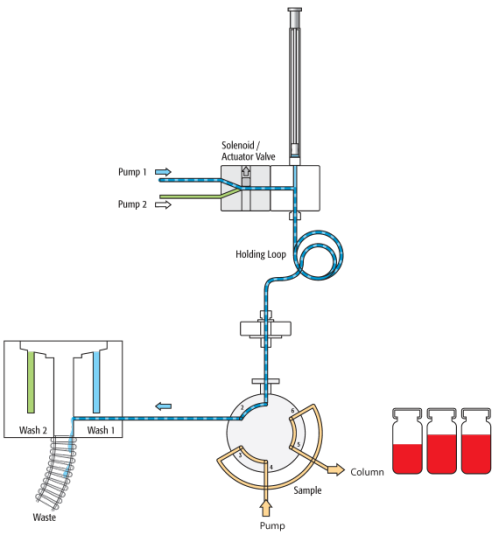

Figure 48.

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Standard: Steps 19 - 20
Active Syringe Needle Wash with Wash Solvent 1.

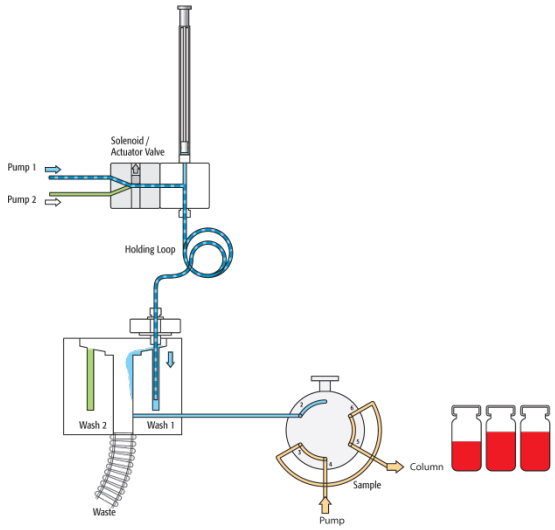

Figure 49.

Standard: Cycle End

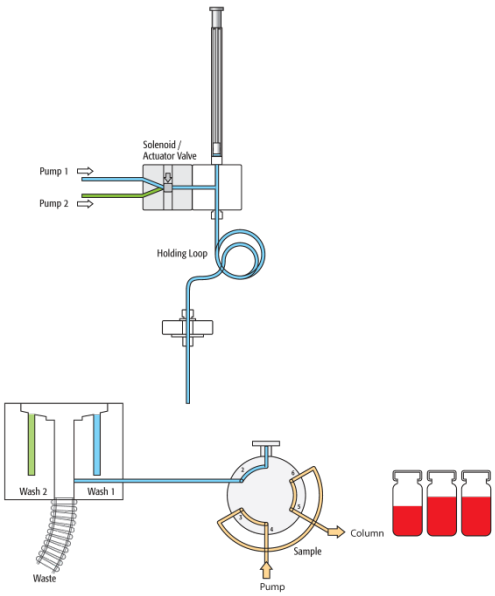

Figure 50.

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5.2. Additional Valve Toggle Step to the DLW Standard Cycle

5.2.1. Considerations for additional "Stator Wash" Cleaning Step

The DLW Standard Cycle has the built-in option for the user to toggle the injection valve at the end of the chromatographic run before equilibration of the column to the start conditions.
If the Method variable "Stator Wash" is set to "1", the extra cleaning process for the valve, with "Valve Toggle", becomes part of the standard cycle.
If the Method variable is deactivated (setting "0"), the DLW Standard cycle ends as shown in Figure 50.

The macro (cycle) is written such that the optional valve toggle steps can be executed before re-equilibration of the column. The exact time the valve needs to be switched on has to be synchronized with the chromatographic method by the user (Method variable "Delay Stator Wash"). The two wash solvents are timed by the Method variables "Stator Wash Time Solvent 1" and "Stator Wash Time Solvent 2". After these wash times have elapsed, the valve is switched back to the start position.

Figure 51 illustrates the recommended retention time for "Stator Wash" or "Valve Toggle" times.

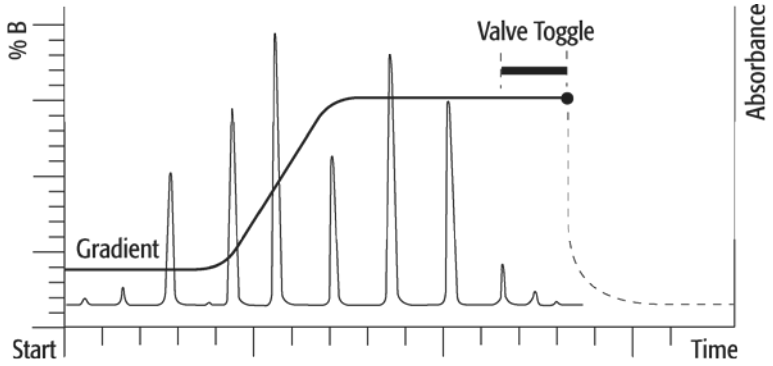

Figure 51. Timing for "Stator Wash" Step

From the chromatographic point of view, it is important to understand the optional cleaning step in detail. It is assumed that the valve stator between ports 1 and 6 (example standard Cheminert Valve) is contaminated and cannot be cleaned during the standard injection process. The valve toggle brings the engraving back between the two ports. Flushing the valve with both wash solvents eliminates any remaining sample material located between stator ports 1 and 6.

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What points need to be considered when using the "Stator Wash" or "Valve Toggle" option?

- Observe the rules if biofluid samples are injected. The first sample contact should always be with an aqueous solution to avoid protein precipitation. After washing with an organic solvent (higher elution power), the system must be flushed again with Wash Solvent 1.

- The first toggle near the end of the chromatographic cycle provides the advantage that the sample loop is already flushed out, first using the mobile phase with a solvent of high elution power (assuming gradient application).

- The second valve toggle time follows immediately after the second solvent flush is completed. A second switching time cannot be programmed. The waiting period before the second valve toggle should be long enough so that the entire system is flushed out by both wash solvents.

  Consider the entire delay volume to determine the second valve toggle time. The DLW internal volumes are:

  - Manifold, 90 µL
  - Holding Loop,108 µL
  - Syringe Needle Gauge 22, 6.7 µL
  - Installed Injection Loop

  Total delay volume: 205 µL + Loop content volume.

- The second valve toggle (back to starting conditions) should be done before the system equilibration time has started. The Loop contents should ideally be a solvent of a low elution power when switched back.

- If isocratic chromatography is applied, it is possible that the remaining contaminants are washed into the system and can build up higher background noise for the column and/or detector over a longer period of time.

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5.2.2. Additional Cleaning step "Stator Wash" or "Valve Toggle" Step-by-Step

Figures 52–57. Additional Cleaning Step "Stator Wash", Step-by-Step.

Stator Wash: End of Standard Injection Cycle,
Start for additional Cleaning Step "Valve Toggle".

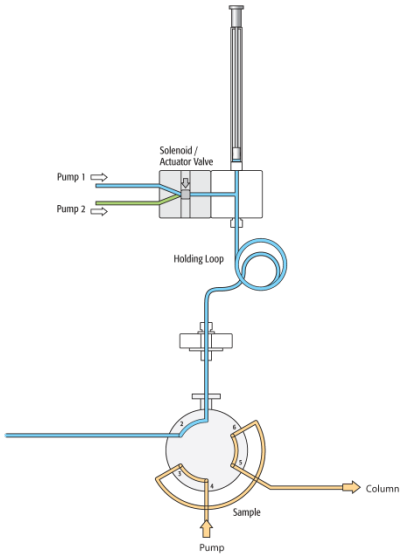

Figure 52.

Stator Wash: Steps 1 - 2
Valve Switched to Load Position (toggle),
Valve clean with Wash Solvent 1.

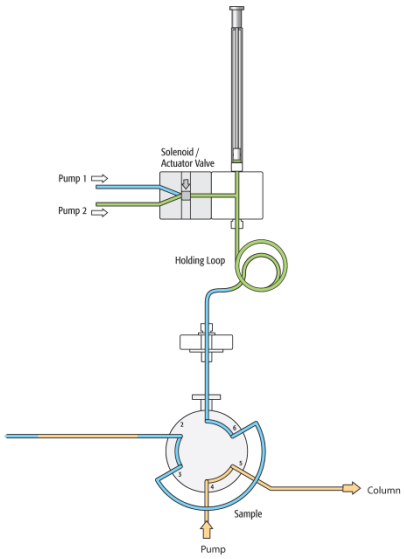

Figure 53.

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Stator Wash: Step 3
Valve Clean with Wash Solvent 2.

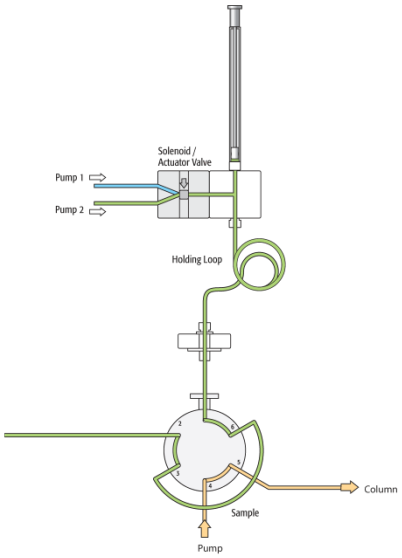

Figure 54.

Stator Wash: Step 4
Wash Solvent 2 dispensed by Wash Solvent 1.

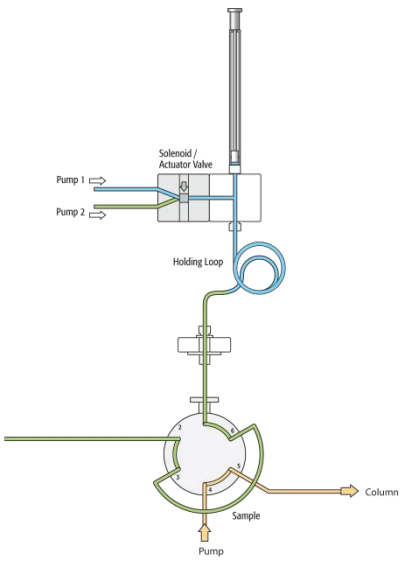

Figure 55.

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Stator Wash: Step 5
Second Valve Clean with Wash Solvent 1.

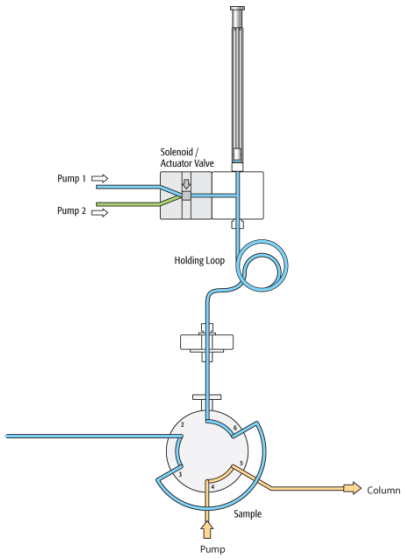

Figure 56.

Stator Wash: Step 6
Valve switched back to Inject Position (toggle).

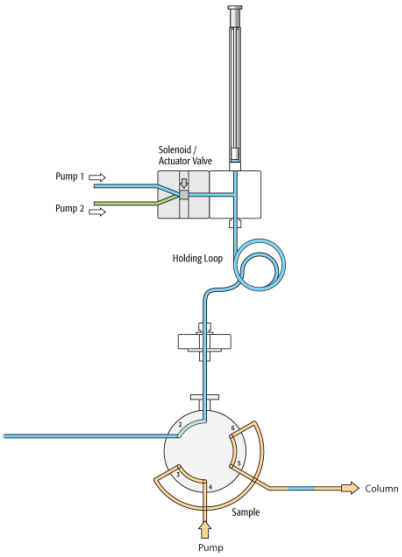

Figure 57.

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5.3. Fast Injection Cycle Step-by-Step

Figures 58-69. The Fast Injection Cycle Step-by-Step.

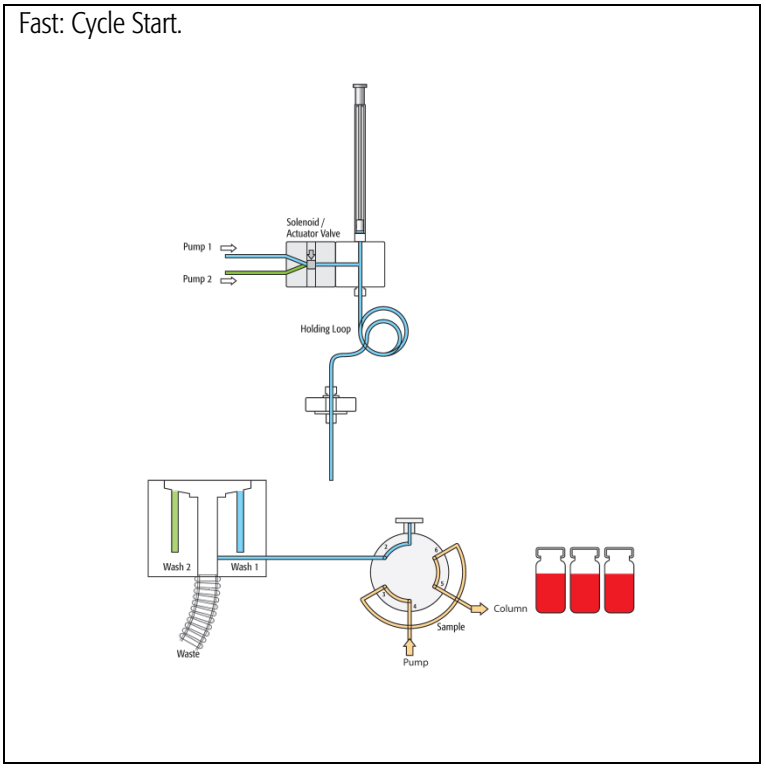

Figure 58.

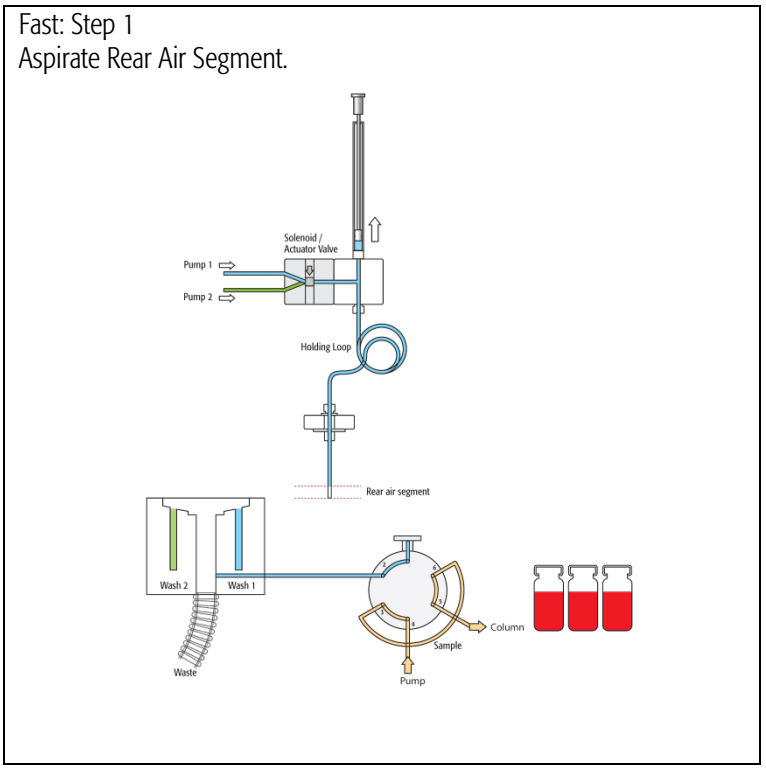

Figure 59.

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Fast: Step 2
Get Sample.
Aspirate Rear, Inject and Front Volume.

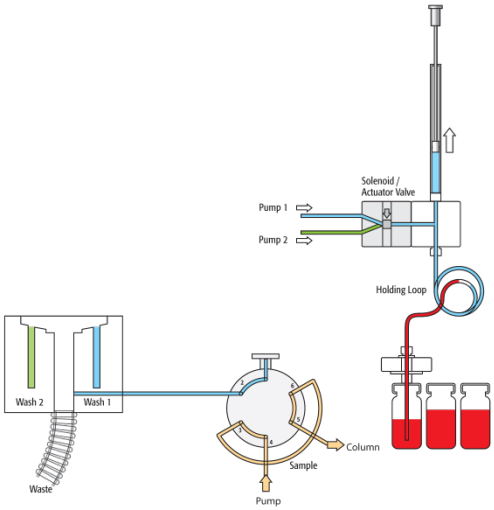

Figure 60.

Fast: Steps 3 - 4
Aspirate Front Air Segment.

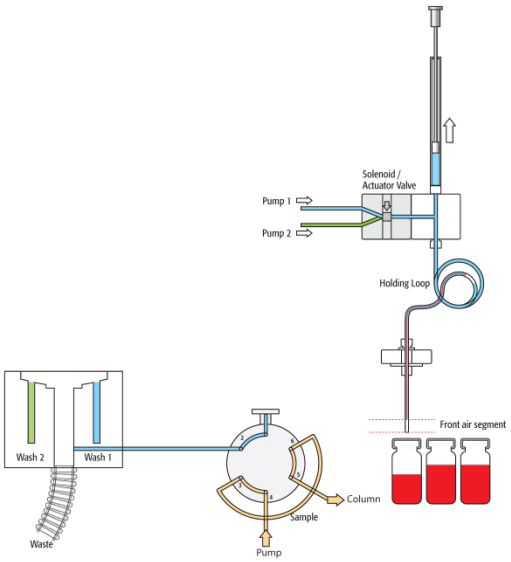

Figure 61.

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Fast: Steps 5 - 6
Dispense Font Air Segment and Front Sample Volume to Waste.

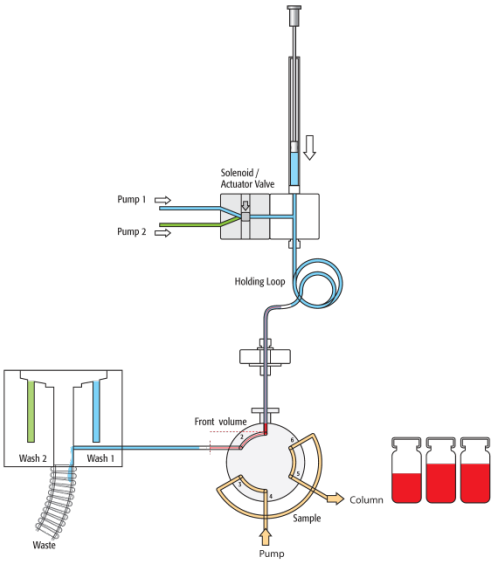

Figure 62.

Fast: Steps 7 - 8
Valve is switched to LOAD position, Loop is filled with "Inject Volume".

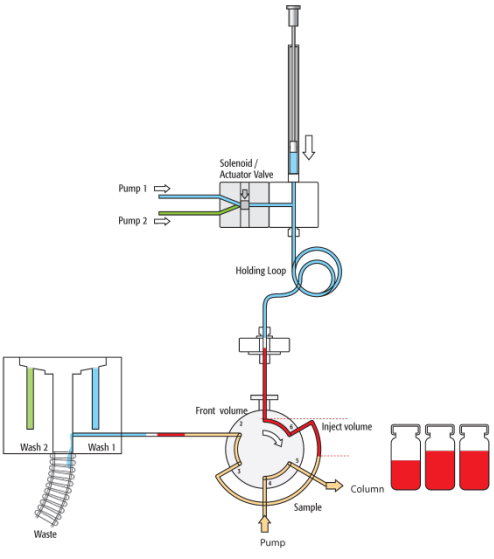

Figure 63.

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Fast: Step 9
Valve is switched to INJECT position, start chromatographic process.

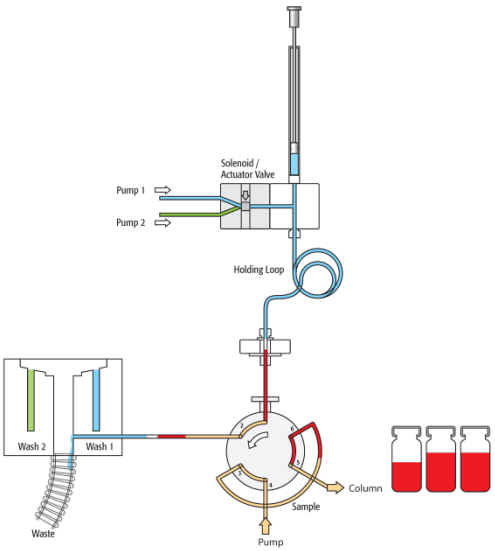

Figure 64.

Fast: Step 10
Rear Sample Volume and Air Segment are dispensed to Waste.

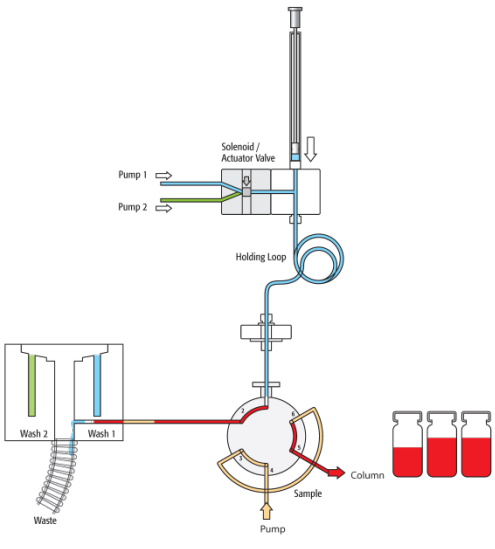

Figure 65.

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Fast: Steps 11 - 12
Valve Clean with Wash Solvent 2.

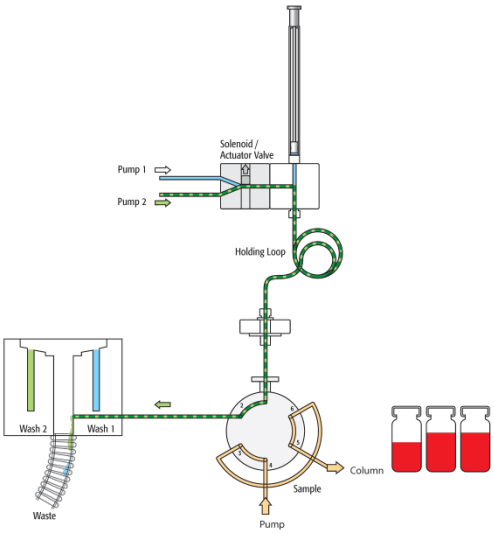

Figure 66.

Fast: Steps 13 - 14
Valve Clean with Wash Solvent 1.

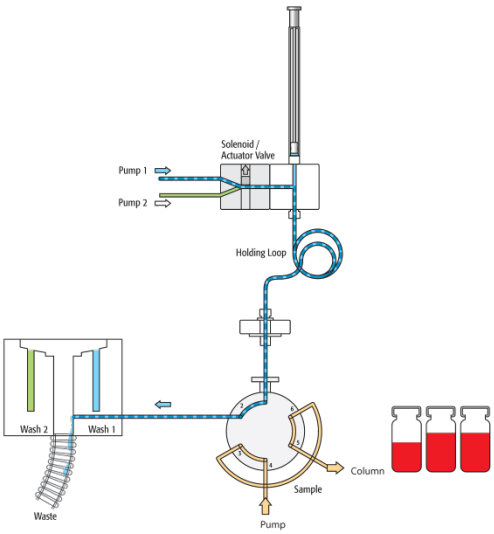

Figure 67.

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Fast: Step 15
Active Syringe Needle Wash with Wash Solvent 1.

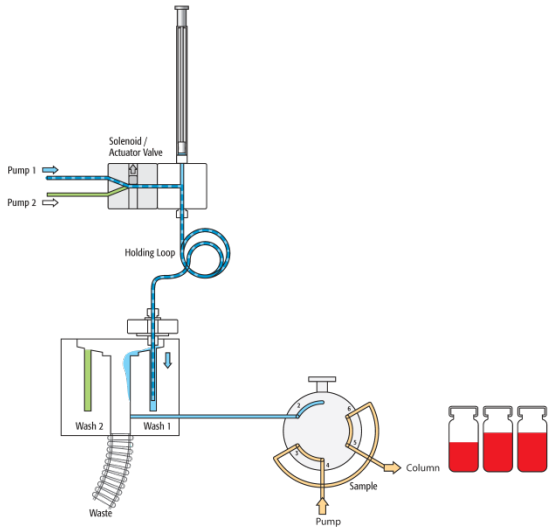

Figure 68.

Fast: Cycle End.

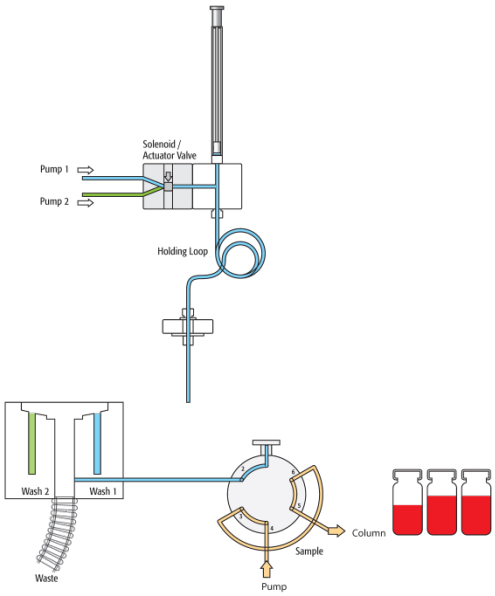

Figure 69.

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H. Troubleshooting

note

The PAL System is always one of several components that make up a complete chromatography system. The following Troubleshooting Guide is limited to the DLW Option only.
Troubleshooting for the PAL System is covered in the corresponding PAL User Manual.

1. Troubleshooting based on observed Symptoms or Error Messages

Symptom or Error Message	Possible Cause	Recommended Action
No or very low detector signal is observed.	Clogged Syringe, Needle or Holding Loop.	Remove syringe and aspirate/dispense liquid manually. Clean syringe and/or needle/Holding Loop.
	Bent needle.	Inspect and/or change syringe.
	No sample liquid injected.	Check and/or adjust Needle Penetration into sample vial (see Section F, point 6.2).
	Sample volume too low.	Increase sample volume.
	The valve needle guide and/or needle seal are not properly installed.	Check valve needle guide and seal (see Section F, point 6.2.5).
	The valve ports are not plumbed correctly to the pump and/or detection system.	Check plumbing connections (see Section F, point 6.2).
	Wrong valve type specified.	Check valve type by selecting the path: Menu/setup/Objects/Injectors/LCVlv1/valve.
Sample is backing up on the valve needle guide.	Syringe OD too small.	Check the syringe needle for correct Gauge. Gauge 22 = OD 0.72 mm
	Valve needle seal leaks.	Change valve needle seal (see Section F. point 6.2.5.)
	The needle penetration depth for the injection valve is not set correctly.	Adjust the Injection Valve Needle Penetration (see Section F, point 6.2.6).
	The Syringe Plunger speed is too high, resulting in excessive pressure in inlet.	Reduce Inject Speed in method.
Syringe does not fill properly.	DLW Actuator/Solenoid not functioning.	PAL Firmware level < 4.0 or PAL Firmware Object for "DLW Option" not loaded onto the system.
		Solvent path blocked. Prime the system or inspect it to identify the reason for clogging.
		Air bubbles trapped in the system, caused by loose connections or tubing not cut square.
	Syringe plunger tip worn out.	Replace syringe plunger. Check if glass barrel is scratched (damaged). If in doubt, replace the entire syringe at once.
	DLW Actuator Control PCB defect.	If possible, verify the DLW Actuator Holder Assy. on another PAL System. If defect is definite, replace PCB.
	DLW Flow Diverter.	Check the Flow Diverter. - Is the Flow Diverter inserted? Inserted in full up position? - Is the solvent flowing freely in the tube?
	Fitting replaceable needle.	Check the fixation and seal of the replaceable syringe needle. - Is the retaining nut tight? - Is the PTFE seal installed? - Is the needle entry free of debris, not clogged? - Is the correct needle diameter used? Gauge 22.

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Symptom or Error Message	Possible Cause	Recommended Action
Sample peaks/responses are not reproducible.	Dirty syringe and/or Holding Loop.	Increase Pst Cln Slv1/Slv2 values in method. Use PreClnSlv1/Slv2 and PreClnSpl.
	Syringe pressure differences.	Increase Pullup Delay value.
	Vacuum created in sample vial.	Reduce sample volume in sample vial. Use setting under "F3-Setup/System/PrePressureVial".
	Method Parameters	Check recommended Method Parameters in Flow Chart "Firmware Overview", Appendices, point 4. The following parameters are crucial and should be verified first: - Fill speed - Pullup delay - Injection Speed - Post Inj Delay *Remark: The parameters mentioned above are listed in the standard injection cycle 'LC-Inj'. Please note that this cycle cannot be used for the DLW operation. However the values of these parameters can be used as a good starting point.*
Excessive carryover between samples.	Loose, instable or wrong connection.	Check all connections within the DLW system, such as needle, Holding Loop, injection loop connections, etc.
	Inappropriate wash solvent(s).	Use appropriate wash solvent(s).
	Waste tubing ID at injection valve is too small, causing waste liquid to be pulled back, by capillary action, into the rotor groove.	Replace the waste tubing with larger ID tubing.
	Air gaps not formed. Liquids of different type diffusing into each other.	Verify the DLW method parameter for "Airgap Volume". Prime the entire system.
Excessive carryover between samples.	Dirty needle, Holding Loop and/or valve injection port.	Increase Pst Inj Slv1/Slv2 values in method (cleaning time). Use PreClnSlv1/Slv2 and PreClnSpl.
	Inappropriate wash solvent(s).	Use appropriate wash solvent(s).
	Waste tubing ID at injection valve is too small, causing waste liquid to be pulled back, by capillary action, into rotor groove.	Replace the waste tubing with larger ID tubing.
	Damaged or grooved valve rotor.	Replace valve rotor, see Section H, point 4.
	Valve needle seal leaks.	Change valve needle seal, see Section F, point 6.2.5.
	Inappropriate wash solvent composition.	Use appropriate wash solvent. Observe also the order of use for biological samples. The first wash is always an aqueous solution followed by organic solvents.
	Tube of Holding Loop at end not cut square or nut/ferrule fixation not correct.	Check the connections from the Holding Loop. - Is the pilot distance appropriate? - Is the tube end damaged? - Is the tube end cut absolutely square? - Are the right nuts and ferrules used?
	DLW Actuator/Solenoid questionable, defect.	Verify the functionality of the DLW Actuator. Is solvent flowing when active? Use command "Utilities/Wash Station" for tests.
	Solvent Frits in wash solvent reservoir blocked.	Clean the frits in an ultrasonic bath with an appropriate solvent. If flow is not ensured after cleaning, replace frits. Replace wash solvent and clean reservoir bottle and tubing carefully.

Table 6. Troubleshooting

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2. Special Considerations for Troubleshooting

2.1. Tubing Connections

Special attention must be paid to all tubing connections. Common mistakes in routine practice are:

- Nut and ferrule not connected tightly and leak
- Pilot distance incorrect
- Tubes not cut square
- Wrong type of ferrule or nut used
- Air bubbles trapped

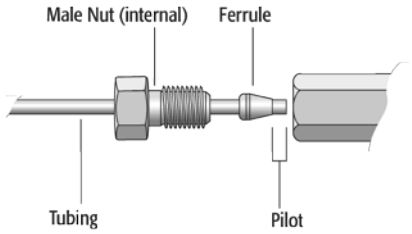

Figure 70. Definition of Terms for Tubing Connections.

Disregarding these basic rules could lead to:

- Dead volume
- Peak deformation or peak splitting
- Carry-over effects
- Decreased resolution of the tubing between column and detector

2.1.1. Nut and Ferrule Tightening

2.1.1.2. Stepwise Tightening

The first important rule when tightening a nut and ferrule is to proceed stepwise:

1. Tighten the nut by hand as much as possible.
2. Continue to tighten using a wrench until resistance is felt. Add one or two quarter turns to reach the sealing point.
3. Open the connection and remove the assembly to inspect the pilot distance, see Fig. 70 above.
4. Install the assembly again and tighten the nut manually at first. Use the wrench to reach the same sealing or resistance point as in the step before and add one extra quarter turn to obtain the final seal.
5. Check the seal when liquid is pumped through the system. In case a leak is observed, tighten once more by another quarter turn. The rule is to always go from leaking to properly sealed, and never try to release an over-tightened seal.

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Do not over-tighten the nut/ferrule. If a connection proves to be leaky, use another quarter turn to tighten. Step-by-step tightening is the correct approach. Over-tightening even once will damage the seat and the next connection can only be sealed by force. If the seat is damaged, there is a considerable chance that the ferrule will stick and cannot be removed without force or special tools (such as a drill).

If different materials are used, such as polymer tubing and nut together with a stainless steel ferrule, then over-tightening is always a danger. The sst ferrule will bite into the polymer tube and block the flow.

When installing a tube with a narrow bend, typically a loop, it is advisable to fix one connection first without inserting the second nut into the female counterpart. After tightening, open the connection. Start preparing the second connection without inserting the first nut. Open the second connection again and check the pilot distance. The last step is to insert the complete tubing with the two connections and make a final seal as described above.
This stepwise installation of a bended tube is mandatory for PEEKsil tubing (fused silica tubing sheathed with PEEK polymer).

2.1.1.2. Re-use of Installed Nuts/Ferrules

The second rule to be observed is: Do not re-use an installed nut/ferrule for any other connection.

Any tube with an installed nut/ferrule used for one purpose should not be installed for any other application. Cut a portion of the tube end until straight tubing is reached and remove the ferrule.
This rule also applies to loops. Never re-use a loop with another valve or a valve after exchanging the stator.

If a finger-tight nut has been installed, replace it as well. If a stainless steel nut has been used, ensure that the correct type is used; see details below. Newly installed finger-tight ferrules can hold a backpressure of up to 200 bar. This remark is valid for the well known 'finger tight nuts' which are used since a long time. Recent developments try to overcome this deficiency. Please observe the market to find a technical suitable solution.

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2.1.2. Pilot Distance Incorrect

When making a new tube connection it is critical to follow these guidelines:

1. Slip the nut and ferrule over the tube. The end of the tube should stick out approximately 50 mm (2 inches).

2. Press the tube end firmly into its female counterpart for the connection.

3. Move the nut and ferrule together into the female counterpart for connection. At this stage is it important to maintain pressure on the tube to avoid the tube slipping backwards, out of position.

4. Tighten the nut as described above.

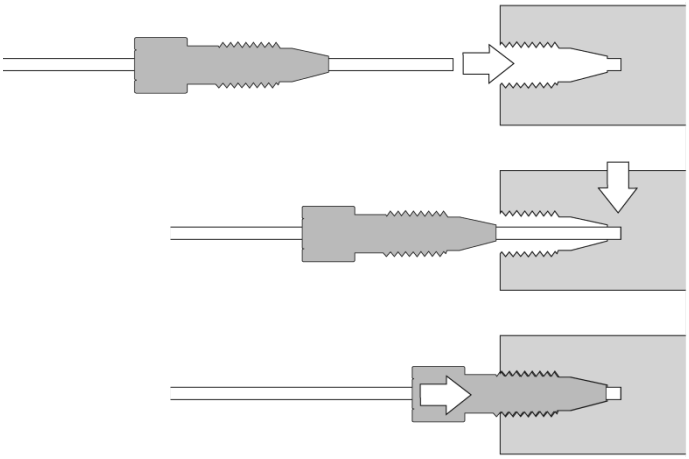

Figure 71. Pressing Tube into Place.

Failure to follow this procedure will lead to an incorrect pilot distance, which will result in actual dead volume (not delay volume).

2.1.3. Tube Not Cut Square

All tubes must be cut absolutely square. Any deviation from square causes a dead volume which yields carry-over and other chromatographic effects. This applies to stainless steel, PEEK or Polymer tubing. It is important to use the correct tool for each material when cutting a tube.

Stainless steel tubes are often cut using pliers. Often, an egg-shaped profile will result, which will no longer seal and will cause dead volume. Dedicated pliers for HPLC tubing are available; nevertheless, it is recommended to use precut tubing which is cut smooth, clean and passivated.

Polymer tubes, such as PEEK, Teflon, PFA, etc., are soft and entice the user to cut them with any handy tool. Commercially available tubing cutters from

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many manufacturers are available. If the blade does not provide a clean, right-angle cut, use a different technique or tool.

The best and most commonly-used method for cutting tubing of any material is to use a cutter with an adjustable blade. Carry out the initial turns, readjust the blade a little, turn once more and adjust again until approximately half of the tubing wall is cut. Hold the tube on both sides of the cut with flat-nose pliers and twist the tube until it breaks. For polymer tubing, the same can be done by replacing the flat-nose pliers with tweezers with a flat tip.

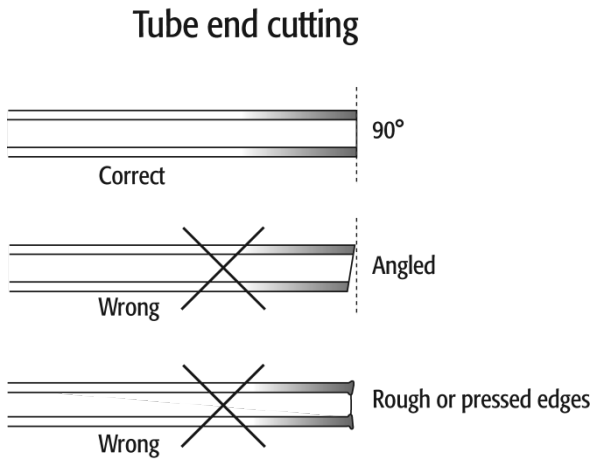

Figure 72. Illustration of various tubing cuts.

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2.1.4. Wrong Nuts or Ferrules used

Do not use a nut or ferrule from a vendor other than specified for the product. Figure 73 below illustrates the resulting dead volume that occurs when an incorrect type is used for connecting.

The second illustration (Figure 74) shows the various forms of the ferrules from different manufacturers. Be aware that some of them are very similar in shape but not similar enough to exchange without risk.

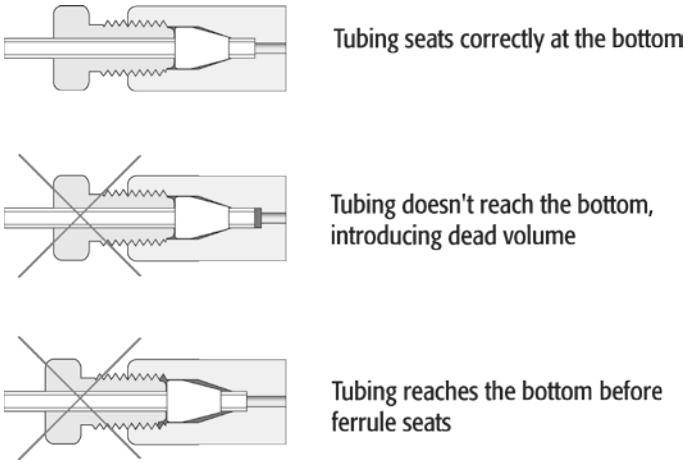
Tubing seats correctly at the bottom
Tubing doesn't reach the bottom, introducing dead volume
Tubing reaches the bottom before ferrule seats

Figure 73. Dead Volume created due to wrong Ferrule Type.

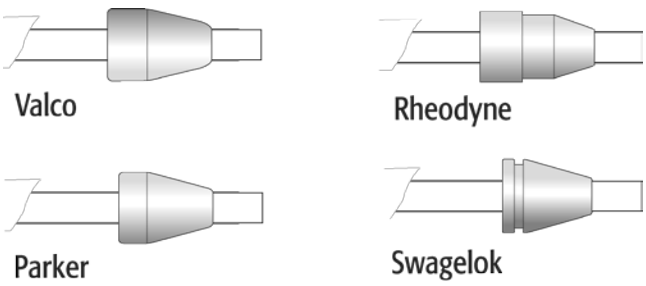

Figure 74. Ferrule Types from various Vendors.

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2.1.5. Avoiding Trapped Air Bubbles

It is common practice to make a connection only if the ports are wetted. The liquid helps to prevent trapped air bubbles which are often very difficult to remove or work out of a system.

2.2. Solvent Delivery Pumps are not priming

It has been observed that the solvent delivery pumps of the "Pump Module" can fail to prime at initial operation.
The pump has to be wetted at the solvent inlet port, or preferably prime the tubing from the Solvent reservoir to the pump and make the connection.
Dry running the pump for a short time will not harm it.

2.3. Solvent Delivery Pump is not functioning

The pump flow rate depends greatly on the viscosity of the selected wash solvent and the given backpressure of the entire system. The valve bore size (standard Cheminert valve 0.25 mm) must be considered, as well as the tubing and injection loop internal diameter.
When in doubt about the delivered volume of a pump, a simple test can provide the answer. Execute the test first, and only if the result is negative is it necessary to check the flow from the pump directly.

- **Test 1:** Pump flow delivered throughout the entire system:

1. Prepare a graduated cylinder with a volume of 10 or 20 mL.
2. Test solvent: Water.
3. Select the following path from the local Terminal:

   Menu/Utilities/Wash Station/select the desired Wash Station

4. The delivery pump and the DLW Actuator are activated; the delivered flow can be collected at the DLW syringe needle.
5. Expected result: A volume of approximately 8 mL should collect within 30 seconds. (Water, DLW syringe needle Gauge 22).

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- **Test 2:** Pump flow delivered at pump outlet:

1. Disconnect the outlet tube from the pump module.
2. Connect PEEK tubing with the following dimensions to the outlet of the pump module: Length 85 mm, ID 0.25 mm (0.010 “).
3. The wash solvent in the reservoir bottle should be water, if necessary, replace the solvent.
4. Activate the pump by selecting following path:

   Menu/F3-Setup/Objects/Events/Pwr-Out1 or Pwr-Out2

5. Collect the pumped water in a graduated cylinder.
6. Expected Result: Within 30 seconds the pump should deliver approximately 10 mL.

This short test will give an indication of whether the system is performing as expected. If none or very little flow is observed, perform a systematic check using a process of elimination. Consult the table in the “Troubleshooting” section for more details.

2.4. Verify the Functionality of the DLW Actuator/Solenoid

The DLW Actuator is electrically connected to the circuit of the heated syringe. Increasing the heated syringe temperature does activate the DLW Actuator. The green LED is the indication that current is applied but please note that this is not proof that the DLW Actuator opens the seat to allow a solvent flow. To check the functionality, the following two tests are described:

- **Test:** Electrical activation of the DLW Actuator

1. Select the DLW syringe by following path and change the standby temperature to 65 °C.
   Path (from local terminal):

   Menu/Utilities/Syringe/Standby Temp/ select 65 °C

2. Expected result: The green LED on the Syringe Holder assembly is turned ON. (Please do not reset the temperature if the second test is activated as well).
   In case this result is not observed, verify the electrical connection from the DLW Actuator to the PCB.

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- **Test 2:** Verify the seal tightness of the DLW Actuator

1. Close the DLW Actuator by setting the standby temperature to 35 °C.
   Use the following path (from local Terminal):

   Menu/Utilities/Syringe/Standby Temp/ select 35 °C

2. Expected result: The green LED on the Syringe Holder assembly is turned OFF.

3. Place a beaker or other adequate vessel under the DLW Syringe Needle.

4. Select the Power-Out signal and activate both DLW Pumps.
   Path:
   Menu/F3-Setup/Objects/Events/PwrOut1 for Pump1 , or
   'PwrOut2' for Pump2

   Activate both pumps by changing the status to the 'Active State'.
   Active State OFF = Pump is activated, start.
   Active State ON = Pump is stopped.

5. Expected result: The DLW Actuator has been closed (step 1). Activating the DLW Pumps delivers solvent up to the point of the DLW Actuator. No solvent should pass the DLW Actuator which verifies the tightness of the seal.

6. In case a solvent flow is observed during the test, remove the DLW Actuator and check if particles are breaking the seal.
   If nothing special is observed, replace the device.
   The PNo. is listed in the Appendix.

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3. Standard Chromatographic Tests

In this section standard chromatographic tests for “carry-over” and “repeatability” are described. These tests should provide basic information for support technicians and users.

The tests described are examples only and should be adjusted accordingly if the configuration of the HPLC system changes.

The test is designed for PAL standard valves with a bore size of 0.25 mm, which allows a flow rate range of 10 to 500 µL/min.

For both the carry-over and repeatability tests, conditions will be described for UV detection.

Details are listed below. Note that the given levels to reach for carry-over and repeatability do not reflect the specification values. The tests described here should give the operator a confirmation whether the system is working as expected. These tests are comparable to an OQ (Operational Qualification) Test.

The test procedure is written for standard equipment, such as a standard syringe of 100 µL and a loop size of 20 µL. The procedure does allow configuring the system with different equipment, but the user has to bear in mind certain limitations. For example, if a 100 µL syringe is installed alongside a loop with a volume < 10 µL, the reliability of a partial loop injection is questionable. The relative standard deviation will be above the expected limit of 1.20%. Example: 2 µL loop, 1 µL partial loop filling yields a relative standard deviation of 2 to 4%. Under such conditions, a full loop injection is the only valid test.

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3.1. Carry-Over Test with UV-Detection

- Test Sample: Chlorhexidine, 0.6 mg/mL dissolved in Water : Acetonitrile = 90 : 10 + 0.1%TFA
- Blank Solution: Water : Acetonitrile + 0.1%TFA

- HPLC System:
  - Binary Solvent Delivery Pump
  - UV-Detector, Wavelength: 257 nm
  - HPLC Column: C18 (Halo), 2.1 x50 mm; spherical 2.7 µm (or equivalent)
  - Column Temperature: Ambient
  - Mobile Phase: A: Water+0.1%TFA; B: Acetonitrile +0.1% TFA
  - Gradient: 10% A hold for 0.5 Min
    to 90% B in 7.5 Min
    hold for 1 Min
- Wash Solvents: Wash1: Water +0.1% TFA
  Wash2: Acetonitrile +0.1% TFA
- Loop Size: 2 µL; Loop ID 0.13 mm
- Injection Valve: VICI/Valco C72VX-1696D-CTC
  bore size 0.25 mm

- Cycle Composer Macro or Cycle (ICC-CE): DLW Standard

- Cycle Composer Method Parameter Settings

Parameter	Value	Parameter	Value
Syringe	100 µL DLW	Pullup Del	500 ms
Sample Volume	7 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/s
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	2 s
Fill Speed	5 µL/s	Post Clean Time Solvent 2	2 s
Fill Strokes	0	Valve Clean Time Solvent 1	3 s
Stator Wash	0	Post Clean Time solvent 1	2 s

Table 7. Carry-over Test with UV-Detection.

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3.2. Repeatability Test with UV-Detection

- Test Sample:
  - Loop Overfill: Caffeine, 520 mg/L
  - Partial Loop: Caffeine, 52 mg/L dissolved in Water : Acetonitrile = 90:10 + 0.1%TFA

- HPLC System:
  - Binary Solvent Delivery Pump
  - UV-Detector, Wavelength: 273 nm
  - HPLC Column: Zorbax SB-C18, 2.1 x50 mm; spherical 1.8 µm (or equivalent)
  - Column Temperature: Ambient
  - Mobile Phase: Water : Acetonitrile = 90:10 +0.1% TFA
  - Gradient: isocratic for 2 Min
- Wash Solvents: Wash1: Water +0.1% TFA
  Wash2: Acetonitrile +0.1% TFA
- Loop Size: 2 µL (Loop overfill, 7 µL); Loop ID: 0.13 mm
  20 µL (partial Loop, 10 µL); Loop ID 0.25 mm
- Injection Valve: VICI/Valco C72VX-1696D-CTC
  bore size 0.25 mm

- Cycle Composer Macro or Cycle (ICC-CE): DLW Standard

- Cycle Composer Method Parameter Settings

Parameter	Value	Parameter	Value
Syringe	100 µL DLW	Pullup Del	500 ms
Sample Volume	7 / 10 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/S
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	2 s
Fill Speed	5 µL	Post Clean Time Solvent 2	2 s
Fill Strokes	0	Valve Clean Time Solvent 1	3 s
Stator Wash	0	Post Clean Time solvent 1	2 s

Table 8. Repeatability Test with UV-Detection.

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3.3. Carry-over and Repeatability Tests with UV-Detection as described for the OQ/PQ/PV Test

- Test Sample: Benzophenone 50 µg/mL dissolved in Water : Methanol = 50 : 50
- Blank Solution: Water : Methanol = 20 : 80

- HPLC System:
  - Binary or isocratic Solvent Delivery Pump
  - UV-Detector, Wavelength: 258 nm (10 mm path length)
  - HPLC Column: C18 , 2.1 x50 - 100 mm spherical 2.7 to 5.0 µm (or equivalent)
  - Column Temperature: Ambient to 30 °C
  - Mobile Phase: A: 20 % Water; B: 80 % Methanol
  - Isocratic: 0.5 to 1.0 mL/min
- Wash Solvents: Wash1: Water : Methanol = 50 : 50
  Wash2: Water : Methanol = 50 : 50
- Loop Size: 20 µL; Loop ID 0.25 mm
- Injection Valve: 6-port Injection valve with bore size 0.25 mm or 0.40 mm (Example: VICI/Valco C72VX-1696D-CTC)

- Cycle Composer Macro or Cycle (ICC-CE): DLW Standard

- Cycle Composer Method Parameter Settings

Parameter	Value	Parameter	Value
Syringe	100 µL DLW	Pullup Del	5000 ms
Sample Volume	60 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/s
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	6 s
Fill Speed	2 µL/s	Post Clean Time Solvent 2	6 s
Fill Strokes	0	Valve Clean Time Solvent 1	6 s
Stator Wash	0	Post Clean Time solvent 1	6 s

Table 9. Test with Benzophenone and UV-detection.

Expected results for the Benzophenone test are given below.
The OQ/PQ/PV procedure defines the following limits:
- Repeatability : 0.8 % rel. standard deviation
- Carry-over: ≤ 0.10 %.

These limits can easily be reached with an intact system using the described Benzophenone test. Typical values may be:
- Repeatability: 0.3 to 0.5 % rel. standard deviation
- Carry-over: ≤ 0.030 %.

If the values are significantly higher, it is necessary to troubleshoot the system. For hints, see the section on 'Troubleshooting'.

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I. Replacing Parts

note

Safety Warning:
Before beginning any maintenance work, always disconnect the power cord(s) from the power supply or from the various power supplies if optional devices are installed.
Capacitors inside the instrument can remain charged, even if the instrument is turned off.

To avoid damaging electrical parts, do not disconnect an electrical assembly while the power to the PAL system is on. Once the power is turned off, wait approximately 30 seconds before you disconnect an assembly.

This section provides a short description on parts that can be replaced in the field. Only basic instructions are given. Additional details will be provided with an update of the addendum.

The parts, along with ordering information, are listed in the Appendices, point 3.

1. DLW Pump Module

The following parts (keywords) from the DLW Pump Module can be individually exchanged:

- Single Pump Module (incl. electrical connection)
- Solvent Tubing Kit
- Electrical cable (from Pump Module to PAL System)
- Pump control PCB
- DLW Tubing Kit

2. DLW Wash Station

The following parts (keywords) from the DLW Wash Station Module can be individually exchanged:

- Wash Station Block
- Wash station Inserts
- Waste Tube
- Waste Tube (Valve-to-Wash station)
- Dummy plugs

note

The inserts and individual parts of the Wash Station Block are not yet available. This will change with future updates.

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3. DLW Syringe

The following parts (keywords) from the DLW Syringe assembly can be individually exchanged:

- DLW Syringe 100 µL
- DLW Syringe Plunger (for syringe 100 µL)
- DLW Syringe Plunger Holder
- DLW Syringe Retaining Nut
- DLW Syringe Replaceable Needles

4. DLW Syringe Holder Assembly

The following parts (keywords) from the DLW Syringe Holder assembly can be individually exchanged:

- DLW Syringe Holder Assembly (complete assembly)
- DLW Needle Adapter Block
- DLW Needle Holder Assembly
- Holding Loop
- DLW Manifold
  (Entire unit assembled, consisting of DLW Solvent Selector, DLW Syringe Adapter with Perfluor O-ring installed.)
- DLW Flow Diverter
- DLW Actuator/Solenoid
- DLW Actuator Control PCB (incl. cable)
- DLW Syringe Holder Fixation Screw

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J. Maintenance

Regularly performing maintenance helps ensure the accuracy and precision of the PAL System. Suggested intervals for maintenance procedures are given below to ensure uninterrupted operation.
If you use the system extensively (for example, throughout nights and weekends), or if you use corrosive solvents, you may need to perform the maintenance procedure more frequently.

The points listed below are dedicated to the DLW Option. Maintenance for the PAL system is listed in the specific PAL User Manual.

Maintenance Step	Interval
Prime the entire DLW system by using the 'Utilities' functions 'Wash Stations' or 'Syringe'.	Daily or as needed. Required before routine running.
Clean the outside of the instrument. Use only a soft lint-free cloth dampened with mild soap and water.	Weekly or as needed.
Clean Instrument, syringe and surfaces.	Weekly or as needed.
Replace the DLW Syringe plunger.	The Syringe Plunger for a gas tight syringe (polymer tip) has to be replaced on a regular basis. The interval is highly dependent on the application, throughput, quality of sample solution (particles, etc.) and washing solvent. It is advisable to check the tightness of the plunger on a regular basis to gain experience with the application.
Replace the DLW Syringe Needle. Observe the correct placement of the Teflon seal.	Only as needed (e.g. if bent).
Replace the Valve Needle Seal.	Check the tightness of the needle in the needle seal. See section F, point 6.2.5.
Check all connections for tightness.	Weekly or as needed.
Replace the DLW Holding Loop.	Recommended to be changed once a year as a preventative maintenance step.
Check the spring loaded Needle Holder Assembly for functionality. The spring compression in particular must be checked.	Weekly or as needed.
Wash and Waste Solvent reservoirs. Check flow restriction of frits. Quality of solvents, biological growth.	Weekly or as needed.
Check the functionality of the DLW Actuator/Solenoid.	Weekly or as needed. Recommended to be changed once a year as a preventative maintenance step.

Table 10. Maintenance Check List.

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K. Limitations

1. PAL System

The DLW Option can be operated only with a PAL-*xt* system which requires PAL Firmware level 4.1.X and the APR Control-*xt* board.
An existing PAL System can be upgraded to this level. For details contact your CTC Analytics representative.

2. Sample Volume

The current version of the DLW Option can handle sample volumes from 2 to 100 µL.

3. Wetted Parts and Material

Some components are not compatible with certain solvents or chemicals that are used in the given applications. The material for wetted parts of the individual component is described in the section "Installation", Specification" and in the specific Point for this component.

4. Tubing Internal Diameter (ID) and System Backpressure

Any tubing and component in the flow path, such as an injection valve, will contribute to the system backpressure. For example, rather short tubing with an ID 0.13 mm (0.005") can build up high back pressure when standard wash pump flow rates are used.
The wash solvent pumps operate in the range of mL/min. Such a flow rate in combination with a high system backpressure, caused by small ID tubing, will slow down the pump and thus the flow rate.

The above described points are only critical if an HPLC setup dedicated for nano flow is selected.

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L. Appendices

1. Definition of Terms

Job Queue

A Job Queue is a list of sample processing Jobs. Jobs are executed in the order displayed on the JOB QUEUE menu screens. New Jobs may be added to the queue while samples are being processed.

Job

A Job contains the information needed by the PAL System to process multiple samples using the same processing steps. The elements of a Job are a Method and a Tray that define the location of the samples to be processed. Jobs are automatically numbered from 01 to 99 and then restart with number 01 when they are added to the Job Queue.

Cycle

A Cycle consists of the specific operations necessary to process one sample. The Cycle operations are repeated for each sample within a Job. Cycles are designed for specific applications.

Method

A Method defines how the samples are processed. The elements of a Method are a Cycle, a Syringe and a Parameter List. Methods have names with up to eight characters and can be edited, copied, and deleted.

Method Parameters

Method Parameters are associated with the Cycle operations. User-assigned Parameter values define how a processing operation is performed. A zero Parameter value will disable a Cycle operation. Cycle Parameters are application-specific.

Tray Holder

A Tray Holder can hold one or more trays. Each Tray Holder has a reference position (X-, Y-, Z-coordinates) that defines its location.

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Tray

A Tray holds multiple samples. Trays are defined by designating the Tray Type (see below) and the Tray Holder. Tray names are used to identify the sample source within a PAL Job.

Tray Type

A Tray Type defines the pattern and sampling sequence of sample locations within a Tray.

Stack

A Stack is a particular type of Tray Holder that is designed to hold micro-plates. A six-drawer Stack holds 12 standard micro-plates, two in each drawer. A three-drawer Stack holds six deep-well micro-plates, two in each drawer.

PAL Object Manager

Software to load a PAL Object List to an instrument if a Module (hardware module) has been added to the PAL System. In a special mode, Object Manager can also be used to create and maintain Object Lists.

PAL Object List

If a PAL Module (hardware) is added to an instrument, several Objects have to be loaded into the firmware. These Objects are collected in an Object List and stored in a file with the extension "*.pol".
Object Lists are delivered together with Object Manager Software and are grouped into folders for the different kind of Modules (e.g. Syringes, Tray Holders, Valve Drives). The name of an Object List starts with the Module part number with variants added (e.g. first or second Stack). The name of the root folder includes the revision (version) which is dependent on the firmware version (e.g. "PAL-*xt* Object Lists Rev. A" for firmware 4.1.X or higher).

Object Class

Each Object belongs to an Object Class (e.g. Syringes, Trays, Injectors).
The Object Class defines the Items of an Object.

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Object Item

An Object contains several Items which can be numerical values with a physical unit (e.g. X-, Y-, Z-Position, Penetration, Syringe Scale Length, Syringe Volume) or references to other objects.
Note that the term “Parameter” is reserved for “ATOM Parameter” (PAL Firmware commands to be used for a PAL Cycle or Macro).

Objects

Objects are data structures describing the properties of physical modules. Certain modules (e.g. a Stack) require several objects.

Module

PAL hardware module, either part of a standard PAL configuration (e.g. Combi PAL, HTS PAL) or an optional addition (e.g. Cooled Stack, MALDI Tool, Dilutor).
The term “Module” is intentionally used to differentiate from “Object”, which is reserved for the PAL Firmware Object.

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2. Naming Convention

This section recommends the standard naming convention for the PAL System. Following this convention will allow the PAL setup to be pre-configured for certain applications. This will simplify software backups and application development, and will improve technical support and training.

Tray Type	Tray Description
VT200	Vial Tray, 200 positions (10 x 20) For 7 mm micro-vials, 1 mL
VT98	Vial Trays, 98 positions (7 x 14) For 12 mm vials, 2 mL
VT78	Vial Tray, 98 positions (6 x 13) For 7 mm micro-vials, 1 mL (opposite side of 98 positions Tray)
VT54	Vial Tray, 54 positions (6 x 9) For 12 mm vials, 2 mL
VT21	Vial Tray, 21 positions (7 x 14) For 12 mm vials, 2 mL (opposite side of 32 positions Tray)
VT32-10	Vial Tray, 32 positions (4 x 8) For 23 mm headspace vials, 10 mL
VT32-20	Vial Tray, 32 positions (4 x 8) For 23 mm headspace vials, 20 mL
MT96	Standard 96-position shallow microplate
DW96	Deep well 96-position microplate
MT384	High density 384-position shallow microplate

Table 11. Naming Conventions.

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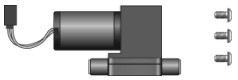
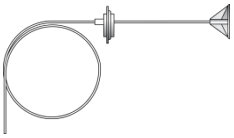
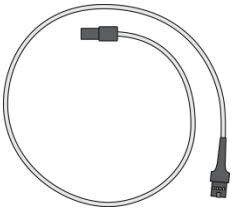
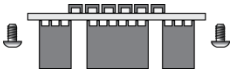
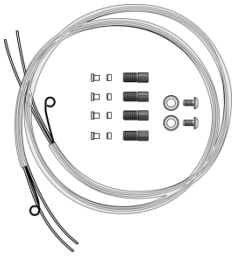
3. DLW Option, Spare Parts and PM Kit

3.1. Spare Parts Order Information

Part No.:	Description	Illustration
DLW Pump Module		
PAL DLWPump	PAL DLW Pump Kit (single module); incl. electrical connections; 24V.	
PAL DilAspKit	Aspiration Tube Kit, consisting of: 1 pc. Tube PFA 1 pc. PEEK solvent filter 10 µm 1 pc. Plug with feed through hole.	
Cbl R4LX-1300	Cable for PAL-*xt* Wash Station round connector, length 130 cm	
PAL DLWPCB	PAL DLW Pump Control Module PCB Kit	
PAL TubeDLW	PAL DLW Wash Station Tubing Kit, Consisting of: 2 tubing, wire supports and connections.	

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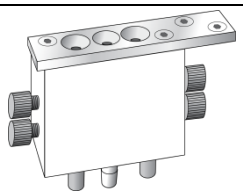
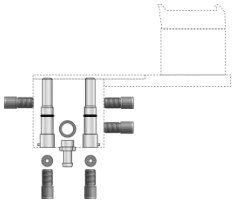
Part No.:	Description	Illustration
DLW Wash Station		
PAL DLWWash	PAL DLW Wash Station Block, complete assembly (incl. centering plate).	
PAL DLW Insert	PAL DLW Wash Station Insert Kit, consisting of: 2 pcs Inserts 1 pc. Waste Connector 5 pcs. Dummy Plugs	
PAL TubeWaste	Tube Waste for Wash Station, length 2 m.	

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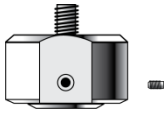
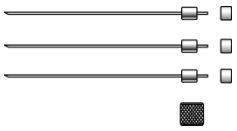
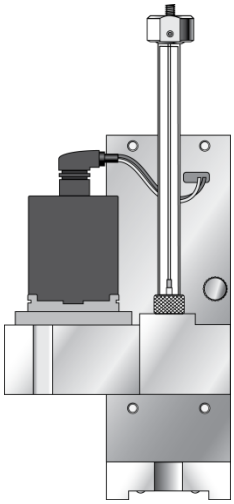
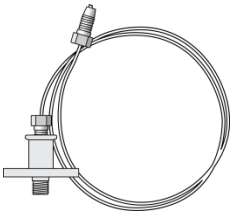
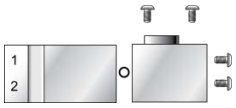
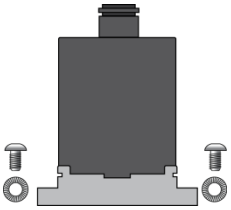
Part No.:	Description	Illustration
DLW Syringe		
SyrC DLW100-R	CTC Syringe for PAL DLW Option, 100 µL gastight; Ø7.7 mm; Scale Length 60 mm; Thread ¼-28 UNF. Removable needle not included.	
PLG DLW 100	Replacement Plunger for Syringe SYRC DLW-R.; pkg of 10.	
PAL DLWPlg	PAL DLW Plunger Holder	
PAL DLWNdl	Needle Kit for PAL DLW Option, Gauge 22 PST 3, length 51 mm (3 pcs. per pack); incl. Needle Retaining Nut.	
DLW Syringe Holder Assembly		
PAL DLWHolder	PAL DLW Syringe Holder Assembly; complete Assembly with 100 µL syringe and one syringe needle installed.	
PAL DLWLoop	Kit PAL DLW Holding Loop with Needle, Adapter mounted.	
PAL DLW Manifold	PAL DLW Manifold Kit, complete, assembled.	
PAL DLWFlwDiv	PAL DLW Flow Diverter Pkg of 1.	
PAL DLWAct	PAL DLW Actuator/Solenoid Kit; incl. screws, without cable.	

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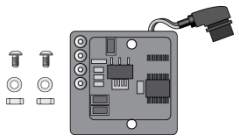
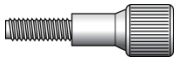
PAL DLWActPCB	PAL DLW Actuator Control PCB Kit; incl. cable and screw set.	
PAL DLW Screw	Syringe Holder Fixation Screw, pkg of 1	
Part No.:	**Description**	**Illustration**
DLW Syringe Holder Assembly (contd.)		
PAL DLW NdlTool	PAL DLW Needle Length Guide Tool, pkg of 1.	
PAL DLWNHA	PAL DLW Needle Holder Kit	

Table 12. DLW Spare Parts.

3.2. PM Kit Order Information

A dedicated PM Kit (Preventative Maintenance Kit) is available for PAL-*xt* Systems including a PAL DLW Option.

This kit contains all parts from the standard PM Kit for HPLC technique (PNo. PAL PM Kit1) and the DLW specific parts.

Packing List PAL PM Kit for HPLC Technique including PAL DLW Option
PNo. PAL PMKitDLW

PNo.	Description
PAL DLWLoop	Kit PAL DLW Holding Loop with Needle Adapter mounted
PAL DLWNdl	Needle Kit for PAL DLW Option, Gauge 22 PST 3, length 51 mm Package of 3 pcs, incl. Needle Retaining Nut
MZ 30-21	Tension Cord 280mm, 1 each
MZ 30-23	Tension Cord, 90 – 95 mm, 1 each
MV 30-13 *	Needle Seal Gauge 22 for Valco Valve, pkg. of 3
Kit SealCap	10 caps and 10 seals for 100-WV solvent bottle
PAL BC TestL	CTC Test Label for Barcode Reader, pkg. of 3
PAL LubKit	PAL Lubrication Kit, incl. brush
BN 4851	Screws for mounting Injection Unit, M4x8, pkg. of 2
PAL PM DocLC	PM Document HPLC Technique Revision F.

Table 13.PM Kit for PAL-*xt* System including PAL DLW Option

* Note: To reorder Needle Seals please use the following information:
PNo.: PAL NdlSeal | Description: Needle Seal Gauge 22 for Valco Valve, pkg. of 10
(Needle Seals for valves made out of PAEK material, Gauge 19, and Rheodyne type valves are available.)

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